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(71) Applicant *(for all designated States except US)*: **GERMAN HUMAN GENOME PROJECT** [DE/DE]; Fraunhofer Patentstelle, Leonrodstrasse 68, 80636 Munich (DE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **WIEMANN, Stefan** [DE/DE]; Grosse Lachstrasse 30a, 69207 Sandhausen (DE).

(74) Agent: **CARPMAELS & RANSFORD**; 43 Bloomsbury Square, London WC1A 2RA (GB).

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(54) Title: HUMAN DNA SEQUENCES

(57) Abstract: Novel human cDNA sequence of a clones, the encoded protein sequence of a clones, antibodies and variants thereof, are provided. The disclosed sequence of a clones find application in a number of ways, including use in profiling assays. In this regard, various assemblages of nucleic acids or proteins are provided that are useful in providing large arrays of human material for implementing large-scale screening strategies. The disclosed sequence of a clones may also be used in formulating medicaments, treating various disorders and in certain diagnostic applications.

HUMAN DNA SEQUENCES

Background of the Invention

Current methods for testing pharmacological substances rely on a three-stage testing approach to drug development. First, candidate compounds are typically screened in some sort of *in vitro* system, like inhibition of cancer cell growth. Candidates are then tested in an animal model, as a first approximation of systemic effects, including efficacy and toxicity. Compounds that still show promise after these initial *in vivo* screens, finally are tested in humans. Again, human testing typically occurs in three phases: toxicity; preliminary efficacy; and efficacy. The entire process can take more than a decade and cost hundreds of millions of dollars. Aside from the monetary costs and protracted time scale, moreover, current testing regimes waste the lives of countless laboratory animals and needlessly endanger the lives of human subjects.

A need exists, therefore, for more sophisticated drug screening techniques that can be done rapidly *in vitro*. These screening techniques ideally will be reflective of systemic and/or organ-specific responses, so that they provide a reliable indicator of action in a human body. Current techniques, however, tend to utilize only a single or limited number of markers, thus answering only very simple questions that are of questionable medical import. For example, a typical *in vitro* assay may ask whether a lead compound binds a particular receptor, which has been implicated in a certain disorder. It is presumed that such binding is indicative of therapeutic usefulness, but it does not even purport to address systemic effects.

Not only are screening techniques for efficacy inadequate, the available toxicity screens likewise are inadequate. Toxicity, on a first level, is usually measured by animal testing. Aside from the complications related to *in vivo* versus *in vitro* testing, such screens are insufficient because of differences in metabolism, uptake, etc., relative to humans. Thus, improved methods would be not only be *in vitro*-based, they would also be more "human."

With the increasing miniaturization of screening assays and the growing availability of targets for pharmaceutical intervention, there is increasing interest in developing arrays containing large numbers of these targets that can be assayed simultaneously. If such an

array contains a large enough population of targets, it can be used to essentially mimic the systemic response. In other words, the array becomes an *in vitro* surrogate for the human body. The more refined the array, the more accurate the predictive capability. In theory, an array could be constructed that can detect all of the known human expression products simultaneously, thereby, providing a very reliable indicator of the human response to a given compound. These arrays offer advantages over the present *in vitro* screening systems in that they can assay large numbers of responses simultaneously. They are superior to animal testing because they are more "human" and, thus, more predictive of human responses.

In order to construct such arrays, however, the field is in need of further human targets. Advantageously, such targets will be provided with additional physiologically relevant information, such as whether the target is expressed in a particular tissue and whether it is related to a known functional class of targets. In this way, the artisan can focus as needed, for example, on tissue-specific effects or target class-specific effects, thereby providing information useful in evaluating efficacy and/or toxicity.

In addition to a need for pharmacological screening targets, there is a need for further pharmacological substances. These substances can be used in the formulation of medicinal compositions and in treating a wide variety of disorders.

The present invention responds to the aforementioned and other needs in the field by providing a population of novel targets useful, *inter alia*, in the profiling and medicinal contexts described above.

Summary of the Invention

It is an object of the invention, therefore, to provide a set of human cDNA clones. Further to this object, the invention provides sequences of human cDNA clones that were isolated from libraries generated from different human tissues.

It is another object of the invention to provide assemblages of targets useful in profiling matrices for screening pharmacological test compounds. According to this object, assemblages comprising different populations of human nucleic acids, proteins and antibodies are provided. In different embodiments, cDNA library-specific assemblages and target-family-specific targets are provided.

It is a further object of the invention to provide a database of human nucleotide and protein sequences. Further to this object, novel human nucleotide and protein sequences are provided in electronic form. In one embodiment, one or more of these sequences is provided in a searchable database.

It is still another object of the invention to provide biologically active target molecules useful in treating or detecting human disorders. Further to this object, the invention provides nucleic acid and protein molecules that have the capacity to affect disease etiology or symptoms or correlate with known disease states. Also further to this object, a database is provided which comprises the disclosed molecules in electronic form.

It is still a further object of the invention to provide polypeptides encoded by the human cDNA clones disclosed herein. Further to this object, the invention provides antibodies and fragments thereof that are capable of binding to a specific portion of these polypeptides.

It is yet another object of the invention to provide pharmaceutical compositions which comprise an effective amount of a pharmaceutical agent, wherein the pharmaceutical agent is selected from the group consisting of one or more polypeptides contemplated by the invention, variants or functional derivatives thereof, and antibodies thereto; and a physiologically acceptable carrier or excipient.

It is still another object of the invention to provide expression vectors comprising one or more human cDNA clones disclosed herein or fragments thereof; and optionally a promoter operably linked to the cDNA clone or fragment thereof. Further to this object, the invention provides methodology for recombinantly producing a desired peptide, comprising expressing in a host cell a peptide encoded by a human cDNA clone disclosed herein.

Detailed Description

The invention results from a need in the art for new human nucleic acids and proteins. This need arises in several contexts. First, there is a need to identify targets for therapeutic intervention. Second, there is a need to identify molecules that may be adversely affected in a therapeutic context, thereby resulting in toxicity. Knowledge of these molecules will aid in

the design of new medicaments with enhanced efficacy and decreased toxicity. Finally, the need encompasses human nucleic acids and proteins that have medicinal applicability in their own right.

In view of these needs, the present inventors set out to isolate and sequence human cDNAs from tissue-specific libraries. In this way, they represent subsets of molecules likely to be targets for therapeutic intervention or for avoiding toxicity. In addition, the inventors divided the molecules into various sub-categories, based on suspected functionality, structural similarity etc, which are of interest from a pharmacological perspective. These molecules are disclosed in provisional application serial nos. 60/149,499 and 60/156,503, filed August 18, 1999, and September 28, 1999, respectively, both of which are hereby incorporated by reference in their entirety.

GENERAL DESCRIPTION OF THE INVENTIVE MOLECULES

The present invention provides novel polynucleotide molecules that, in some instances, have similarities with known molecules. The inventive DNAs were cloned from five different human cDNA libraries. In addition to these DNA molecules, the invention provides their protein translations and antibodies derived from them. The inventive DNA and protein sequences are show individually, below. The inventive nucleic acids also include the complements of these DNA sequences, as well as their RNA counterparts. Methods of producing the molecules also are provided. Further, the invention provides methods for detecting all or part of the molecules and of detecting polynucleotides encoding all or part of the molecules.

The inventive molecules derive from five cDNA libraries: human fetal brain; human fetal kidney; human mammary carcinoma; human testis; and human uterus. For convenience, each sequence bears a designation that indicates from which library it is derived. In particular, these designations are: "hfpbr" for human fetal brain; "hfkd" for human fetal kidney; "hmcf" for human mammary carcinoma; "htes" for human testis; and "hute" for human uterus. The individual libraries were constructed and screened as described below in the examples.

The protein and DNA molecules of the invention are variously described herein as "target" molecules or "inventive" molecules. The sequences and other information pertinent to the nucleic acid and protein molecules of the invention are shown, below.

Interpreting the data disclosed with the Table and cDNA sequences, below:

The table and data below provide the coding sequences of the inventive cDNAs as well as the protein sequences and other useful information, as set out below.

Grouping

The clones were assigned to the following fourteen functional and/or tissue-derived groups:

1. Cell Cycle
2. Cell Structure and Motility
3. Differentiation/Development
4. Intracellular Transport and Trafficking
5. Metabolism
6. Nucleic Acid Management
7. Signal Transduction
8. Transmembrane Protein
9. Transcription Factors
10. Brain derived
11. Kidney derived
12. Mammary Carcinoma derived
13. Testes derived
14. Uterus derived

Description of Clone Files

The individual clone files are structured in the same pattern. The Sections are separated by paragraphs.

1. Clone Name

The clone names are deciphered with reference to the following example: DKFZphfkd2_24e23, wherein the code represents:

- producer of library ("DKFZ") (for convenience, this reference may be eliminated)
- a "p" for "plasmid cDNA library" (for convenience, this reference may be eliminated)
- library name (e.g. hfbr = human fetal brain; hfkd = human fetal kidney; hmcf = human mammary carcinoma; htes = human testes; hute = human uterus)
- an underscore ("_") to separate library information from plate information
- plate number (e.g. "16")
- plate coordinates (letter first; e.g. "f14")

2. Group

3. Introduction

short review of the similarities, function of the protein and possible applications

4. Short Information

specifications about the cDNA (who sequenced, completeness of the cDNA, similarity, who sequenced, chromosomal localisation, length of cDNA, localisation of poly A tail and polyadenylation signal)

5. cDNA-Sequence

6. BLASTn Results

search results of blasting the cDNA sequence against all public databases

7. Medline Entries

information about genes/proteins similar to the novel cDNA (if available)

8. Putative Encoded Protein Information

specifications about the encoded protein (ORF: length and localisation of the reading frame)

9. Protein Sequence

10. BLASTp Results

search results of blasting the protein sequence against all public databases

11. Pedant Information

output of fully automated annotation: summarises peptide information, homologies, patterns as follows:

[Length]

- length of the protein = number of amino acid residues

[MW]

- molecular weight of the protein

[pI]

- isoelectric point

[HOMOL]

- shows protein with closest similarity to the cDNA-encoded protein

[FUNCAT]

- functional information according to a catalogue developed by Munich Information center for Protein Sequences (MIPS)

[BLOCKS]

- Blocks are multiply aligned ungapped segments corresponding to the most highly conserved regions of proteins. The blocks for the Blocks Database are made automatically by looking for the most highly conserved regions in groups of proteins documented in the Prosite Database. The Prosite pattern for a protein group is not used in any way to make the Blocks Database and the pattern may or may not be contained in one of the blocks representing a group. These blocks are then calibrated against the SWISS-PROT database to obtain a measure of the chance distribution of matches. It is these calibrated blocks that make up the Blocks Database. The WWW versions of the Prosite and SWISS-PROT Databases that are used on this server are located at the ExPASy World Wide Web (WWW) Molecular Biology Server of the Geneva University Hospital and the University of Geneva. World Wide Web URL http://blocks.fhcrc.org/blocks/about_blocks.html/ is the entry point to the database.

- here Blocks segments found in the analysed protein sequences are displayed

[SCOP]

Nearly all proteins have structural similarities with other proteins and, in some of these cases, share a common evolutionary origin. The scop database provides a detailed and comprehensive description of the structural and evolutionary relationships between all proteins whose structure is known, including all entries in Brookhaven National Laboratory's Protein Data Bank (PDB). It is available as a set of tightly linked hypertext documents which make the large database comprehensible and accessible. In addition, the hypertext pages offer a panoply of representations of proteins, including links to PDB entries, sequences, references, images and interactive display systems. World Wide Web URL http://scop.mrc-lmb.cam.ac.uk/scop/ is the

entry point to the database. Existing automatic sequence and structure comparison tools cannot identify all structural and evolutionary relationships between proteins. The scop classification of proteins has been constructed manually by visual inspection and comparison of structures, but with the assistance of tools to make the task manageable and help provide generality. Proteins are classified to reflect both structural and evolutionary relatedness. Many levels exist in the hierarchy, but the principal levels are family, superfamily and fold. The exact position of boundaries between these levels are to some degree subjective. Scop evolutionary classification is generally conservative: where any doubt about relatedness exists, we made new divisions at the family and superfamily levels.

- - here SCOPE segments found in the analysed protein sequences are displayed

[EC]

ENZYME is a repository of information relative to the nomenclature of enzymes. It is primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) and it describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided. World Wide Web URL http://www.expasy.ch/enzyme/ is the entry point to the database.

- here EC-number and name of enzymes with similarity to the analysed protein sequences are displayed

[PIRKW]

- functional information according to the Protein Information Resource (PIR) database catalogue developed by Munich Information Center for Protein Sequences (MIPS), the National Biomedical Research Foundation (NBRF) and the International Protein Information Database in Japan (JIPID).

[SUPFAM]

- information according to the Protein Information Resource (PIR) database catalogue of protein superfamilies developed by Munich Information Center for Protein Sequences (MIPS), the National Biomedical Research Foundation (NBRF) and the International Protein Information Database in Japan (JIPID).

[PROSITE]

please refer to 12. PROSITE Motifs

[PFAM]

please refer to 13. PFAM Motifs

[KW]

- overall 2dimensional folding information

- 3D indicates that the proteins is similar to a protein of which a 3 dimensional structure is known

- overall structural information

[]

The last PEDANT-block depicts information about the folding structure of the protein generated by PREDATOR. PREDATOR is a secondary structure prediction program. It takes as input a single protein sequence to be predicted and can optimally use a set of unaligned sequences as additional information to predict the query sequence. The mean prediction accuracy of PREDATOR is 68% for a single sequence and 75% for a set of related sequences. PREDATOR does not use multiple sequence alignment. Instead, it relies on careful pairwise local alignments of the sequences in the set with the query sequence to be predicted.

World Wide Web URL http://www.embl-heidelberg.de/argos/predator/predator_info.html is the entry point to the database.

- H = helix, E = extended or sheet, _ = coil, T = transmembrane, B = beta

- x indicates a low-complexity region with repeat-like structure which is omitted in all BLAST searches

12. PROSITE Motifs

PROSITE is a database of protein families and domains. It consists of biologically significant sites, patterns and profiles that help to reliably identify to which known protein family (if any) a new sequence belongs. World Wide Web URL http://www.expasy.ch/prosite/ is the entry point to the database. A description of the prosite consensus patterns is also provided, below.

13. PFAM Motifs

PFAM (protein families) is a large collection of multiple sequence alignments and hidden

Markov models covering many common protein domains. World Wide Web URL http://www.sanger.ac.uk/Pfam/ is the entry point to the database.

Deposit of Clones

Clones were deposited as a pool with the American Type Culture Collection under accession number _____, from which each clone comprising a particular polynucleotide is obtainable. Each clone has been transfected into separate bacterial cells (E. coli) in this composite deposit.

The clones may also be obtained from the Resource Center of the German Human Genome Project (Heubner Weg 6, 14059 Berlin, GERMANY). The Resource Center library numbers are slightly different that those presented here, but may be readily obtained by the following key or with the assistance of Resource Center personnel.

The library name becomes a number: brain (hfbr2) becomes 564; kidney (hfkd2) becomes 566; mammary carcinoma (hmcf1) becomes 727; testis (htes3) becomes 434;and uterus (hute1) becomes 586. Next, the plate number is converted to two digits (e.g., "2" becomes "02") and is moved behind the plate coordinate, and the underscore is dropped. The following examples are helpful:

Listed Number	Resource Center Number
DKFZphfbr2_16f21	DKFZp564F2116
DKFZphfkd2_1j9	DKFZp566J091
DKFZphmcf1_1c23	DKFZp727C231
DKFZphtes3_14g5	DKFZp434G0514
DKFZphute1_17k7	DKFZp586K0717

The libraries were constructed using two commercially available vectors. The brain (hfbr2 designations) and kidney (hfkd2 designations) libraries utilize pAMP 1 from Life Technologies and are maintained in XL-2Blue (Strategene); the uterus (hute1), testes (htes3) and mammary carcinoma (hmcf1) libraries are constructed in pSPORT1, also from Life Technologies, and are maintained in DH10B (LifeTechnologies). In addition to the following techniques, consultation with the commercial literature available on these clones will make evident all of the housekeeping techniques needed to propagate and isolate the individual constructs. All inserts may be excised with a NotI/SalI digestion. Alternatively, universal primers, flanking the cloning region, may be used to amplify the inserts using PCR methods.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. Methods of probe design are presented below.

Oligonucleotide probes may be labeled with γ-^{32}P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other, non-radioactive labeling techniques can also be used. Unincorporated label typically is removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe can be quantified by measurement in a scintillation counter. Preferably, specific activity of the resulting probe generally should be approximately $4X10^6$ dmp/pmole.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 50 - 100 µg/ml (for XL-2Blue strains 25 µg/ml tetracycline should also be used). The culture should preferably be grown to saturation at 37°C., and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml (for XL-2Blue strains 25 µg/ml tetracycline should also be used)and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them. The filter is then preferably incubated at 65°C. for 1 hour with gentle agitation in 6 x SSC (20 x stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to $1X10^6$ dpm/mL. The filter is then preferably incubated at 65°C. with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2 x SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2 x SSC/0.1% SDS at room

temperature with gentle shaking for 15 minutes. A third wash with 0.1 x SSC/0.5% SDS at 65°C. for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Alternatively, clones may be grown as described above, and PCR used to isolate the insert DNAs. Methods of PCR are described below and are otherwise well known .

ERROR SCREENING

The DNA sequences found herein derive from individual clones, which are publicly available, as noted above. Thus, the skilled artisan will recognize that any specific sequence disclosed herein readily can be screened for errors by resequencing a particular fragment, in both directions (*i.e.*, by sequencing both strands). Alternatively, error screening can be performed by amplifying and/or cloning any of the inventive DNAs, using for example RT-PCR, and sequencing the resulting amplified product. In the event that there is a sequencing error, reference should be made to the deposited clone as the correct sequence.

USES AND BIOLOGICAL ACTIVITIES OF THE INVENTIVE MOLECULES

The inventive molecules and their derivatives are susceptible to a wide variety of uses, based on functional and/or structural properties. The skilled worker will appreciate, based on the biological activities detailed below, and discussed with regard to the individual sequences disclosed below, that the inventive molecules will find usefulness in numerous therapeutic and diagnostic applications.

The DNA molecules, especially the potassium salts thereof, can be used as fertilizer supplements due to their high nitrogen and phosphorus contents. Since the DNAs are of defined length, they are also useful in gel electrophoresis as molecular weight markers. Due to their similarity with known molecules, certain of the DNA molecules and their variants and derivatives may be used in any number of different diagnostic procedures and therapeutic applications. They may also be used to make the encoded proteins.

The proteins themselves have many possible uses. They may be used as a nutritional supplement for humans, animals and even for laboratory use as, for example, medium for bacterial cultures. Moreover, since the proteins are of defined, known sizes, they may be used as molecular weight markers for gel electrophoresis and gel filtration. Because they are of defined sequences, they also have use in microsequencing and protein fingerprinting applications.

Expression Profiling Applications

Given their known tissue expression and functional associations, assemblages of the inventive proteins (or corresponding antibodies) and nucleic acids are particularly suited to expression profiling applications. Expression profiling generally entails constructing an array of indicators that signal the presence of a particular RNA or protein expression product. Such arrays can be used to evaluate, for example, pharmacological effectiveness and toxicity. In particular, expression profiles from such arrays can be generated from cells treated with known compounds, having known properties, and these profiles can be compared to profiles of unknowns to evaluate similarities and differences, which can be correlated with efficacy or toxicity.

Additional uses of profiling include diagnosis, tracking development, and ascertaining signaling and metabolic pathways. For examples of references describing profiling and its uses, see Farr *et al.*, U.S. Patent 5,811,231 (1998); Seilhamer *et al.*, U.S. Patent 5,840,484 (1998); Rine *et al.*, U.S. Patent No. 5,777,888 (1998); WO 97/27317; WO 99/05323; WO 99/09218; and WO 99/14369. For a device for implementing such techniques, see Lipshutz *et al.*, U.S. Patent No. 5,856,174 (1999) and Anderson et al., U.S. Patent No. 5,922,591 (1999).

In one embodiment, a subset of the inventive DNAs will be arrayed on a substrate, like a gene chip, a filter or a 96-well plate. Test samples containing cells are maintained in the presence of a label capable of incorporation into nascent mRNA. Samples are treated with test and control compounds, which will induce mRNA expression in the sample, resulting in incorporation of label. Whole mRNA is isolated and applied to the array such that it hybridizes with the DNAs contained therein. After washing, the amount of hybridization is quantified and a profile is generated. These steps are repeated with various control and test compounds, thereby generating a library of profiles, which can be used to ascertain the relationships relevant to pharmacological efficacy or toxicity.

The matrices used in such profiling, however, need not be limited to those utilizing DNAs. Rather, other nucleic acids, like RNAs and protein nucleic acids (PNAs), as well as the inventive proteins and antibodies corresponding to the inventive proteins may also be employed. Hence, for example, antibodies could form the array and the samples could be treated in order to label nascent proteins. Whole proteins then would be isolated and applied to the antibody matrix. Developing the resulting signal would result in a protein expression profile, which is useful in essentially the same manner as the nucleic acid profile. A protein matrix could be used, for example, in evaluating antibody responses to pharmaceutical agents in order to eliminate possible cross-reactivity.

Moreover, where nucleic acids are used in the matrix, it is often beneficial to use variants (as defined below) of the molecules described herein. This can be used to account for genetic variations that are of little or no consequence to the function of the resultant gene product. Hence, they can account for wobble or conservative amino acid variations that do not perturb function, like variations in some of the protein motifs elucidated below. Thus, each position in the matrix can employ multiple nucleic acid probes that account for a series of variants.

Expression profiling may also be done, in another embodiment, using two-dimensional protein gels in which the inventive proteins are detected. The resultant profiles can be used in the same way as described.

Matrices useful for profiling may be constructed based on different criteria. Of course, the more relevant profiles will take into account expression of most human genes, preferably all of them. In certain situations, however, it is advantageous to look at a smaller subset. For example, if one were concerned about fetal neural toxicity, a fetal brain-specific matrix might be chosen. On the other hand, if one were interested in targeting mammary carcinoma tissue, a corresponding matrix could be used. Thus, matrices may be constructed using all of the sequences available from a tissue-specific library.

* * *

The following discussion relates to some of the various functional and structural groupings that would be of interest to the artisan wishing to construct profiling matrices. Of course, the artisan will also recognized that these functional descriptions may find additional applicability in the therapeutic and diagnostic applications discussed below.

Cell Cycle

A proliferating cell must coordinate replication and chromosomal separation to ensure that the genome is replicated completely, and that a single copy is correctly inherited by each daughter cell. The cell cycle is the coordinated series of events that achieves these aims. Many of the key events are initiated by a family of conserved Seiren/threonine protein kinases, the cyclin-dependent kinases (CDKs), that are activated by the cyclin family of proteins (cyclins A-H). In turn, the cyclin-CDK complexes are modulated by other protein kinases or phosphatases, and by binding specific inhibitor proteins. The enormous variety of ways in which CDK activity can be regulated allows the cell to respond to internal signals generated by preceding events in the cell cycle and to external growth signals.

The somatic cell cycle is divided into four phases: DNA replication (S phase) and chromosome separation (M phase) are separated by gap phases (G1 and G2). At specific control points the decision to begin the next stage (DNA synthesis or mitosis) is carefully regulated.

Cdc2, the primary kinase, is especially required for the G1-S transition and S phase. Cdc4 and Cdc6 are involved at the restriction point, where the cell can decide to proliferate or arrest (G1<->G0) and Cdc7 is a CDK activating kinase (CAK) as well as a subunit of TFIIH.

The Cyclin-CDK complexes are regulated in various ways. One is through phosphorylation by CDK activating kinases (CAK), like the Y15 kinase (Wee1) and dephosphorylation by CDK associated phosphatases (CAP), like Cdc25A a member of the Cdc25 family (Cdc25A, B and C).

An other way of regulation occurs through two classes of CDK inhibitors (CKI), the INK4 proteins p15, p16, p18, and p19, who negatively regulates the cyclin D CDK complexes and second the p21 family with p21, p27, and p57.

The cell cycle is also regulated through ubiquitin-mediated proteolysis involving the destruction of both cyclins and CDK inhibitors by the 26S proteasome, that requires an ubiquitin conjugating enzyme (UBC) and an ubiquitin ligase. The instability is conferred by PEST regions (cyclin D and E) or a ten amino acid region in the amino terminus (degradation box) in the A- and B-type cyclins.

All these modifications play an important role for the cellular localization, because only the nuclear CDK-cyclin complexes are functional for cell cycle. During G1 phase of the cell cycle, cyclines A, E and D are synthesized and bind to their cyclin-dependent kinase (CDK) partners. CDK complexes containing cyclins A, E and D1 are then imported into and concentrated within nuclei. Cdk6- cyclin D3 has been localized to both cytoplasmic and nuclear compartments, although only the nuclear complex is active. As cells enter S phase, cyclin A and cyclin E complexes remain within the nucleus, whereas cyclin D1 relocalizes to the cytoplasm for proteolysis at the onset of S phase. Like Cdk2-cyclin A, Cdc2-cyclin A is nuclear and remains so until it is degraded during mitosis. By contrast, as a result of ongoing nuclear import and more rapid re-export, cyclin B1, which binds to Cdc2 upon synthesis during S phase, is predominantly cytoplasmic. Cdc2-cyclin B2 is also cytoplasmic, although this might occur through anchoring of the complex to some cytoplasmic constituent. At prophase, phosphorylation of cyclin B1 promotes accumulation of Cdc2-cyclin B1 in the nucleus, whereas cyclin B2 remains in the cytoplasm until nuclear envelope breakdown.

Two crucial regulators of Cdc2-cyclin B-Wee1 and Cdc25C exist and are responsible for the G2 to M control point. Wee1 is a nuclear protein throughout the cell cycle, whereas Cdc25C binds to 14-3-3 proteins during interphase and remains predominantly cytoplasmic. In some systems Cdc25C, like cyclin B1, rushes precipitously into the nucleus just before entry into mitosis.

The 110-kDa retinoblastoma (tumor suppressor) protein (RB), a pRB-family member is an important regulator of cell-cycle progression and differentiation. Like the E2F family (E2F1-5) or DP family (DP1-3) of transcription activators, RB suppresses inappropriate proliferation by arresting cells in G1 by repressing the transcription of genes required for the transition into S phase. Before the cell proceeds into S phase, RB becomes phosphorylated at multiple sites by the cyclin dependent protein kinases (CDKs) and loses its transcriptional repressing activity. Phosphorylation of RB during late G1 phase results in the dissociation of the E2F-RB repressor complex which allows S-phase specific genes to be transcribed. Cyclin E is the evolutionary conserved target for E2F and interacts together with CDC2 in late G1.

For a proliferating cell it is vital that only undamaged DNA is replicated because if DNA damage is substantial, its replication can lead to chromosome loss or rearrangement.

Thus, we find a G1<->S checkpoint in late G1 that requires tumor suppressor p53. A p53-dependent G1 arrest is effected by the cyclin dependent kinase inhibitor p21 through higher expression levels that inhibits almost all cyclin CDK complexes.

The kinase responsible for phosphorylating the unidentified kinetochore component in metaphase may be a member of the MAP kinase family and appears to be the proto oncogene c-MOS, a cytostatic factor (CSF) in meiosis.

Several categories of proteins are coded for by clones of the invention within the overall group of "Cell cycle"and include, among others, the following:

Tumor suppressors (e.g. N33): Tumour-suppressor genes are known to be involved in the control of cell growth and division, interacting with proteins which control the cell cycle. The N33 gene is significantly methylated in tumour cells, a mechanism by which tumor-suppressor genes are inactivated in cancer. The N33 gene has been reported by OMIN OMIN (Online Mendelian Inheritance in Man at http://www.ncbi.nlm.nih.gov/htbin-post/Omin) to be associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) prostate cancer suppression (OMIN *601385). Clones in this category include: fbr2_2k14.

C-TAK1 Cdc25c associated protein kinase: Cdc25C is a protein kinase that controls entry into mitosis by dephosphorylation of Cdc2. Cdc25C function is regulated by phosphorylation, too. Serine 216 phosphorylation of Cdc25C mediates the binding of 14-3-3 protein to Cdc25C. C-TAK1 (Cdc twenty-five C associated protein kinase) phosphorylates Cdc25C on serine 216 in vitro. Alterations in the gene coding for the above protein kinase has been reported by OMIN to be associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with Pancreatic cancer (OMIN *60278). Clones in this category include: tes3_7j3.

Cell structure and motility

One of the major differences between prokaryotes and eukaryotes is the ability of the eukaryotic cell to adopt very different shapes dependent on its function during the differentiation process. Animal cells vary from being round to extended cylindric forms like motorneurons or muscle cells. In humans, more than 100 different cell types can be distinguished, each having a characteristic shape. The form of a cell often is closely related to

its capacity to move. Some completely differentiated cells like fibroblasts can still change their form actively, thereby migrating. Other cell types serve as motor elements - "macroscopically" like muscle cells or "microscopically" like ciliated epithelia. Such tasks are fulfilled by a big class of proteins; on the one hand responsible for maintenance of cell structure and contacting neighbor cells or the intercellular matrix and on the other hand for cell motility. These topics cannot be regarded separately: The motility apparatus e.g. must be fixed in the cytoskeleton. Three different types of filaments can be distinguished: Actin filaments, tubulin filaments and intermediate filaments, each present in almost all types of cells.

Actin filaments (F-actin) are built up of monomers (G-Actin). In muscle cells, actin, myosin, for both of which several paralogous genes are known, as well as many more proteins are constituents of the contractile apparatus.

The "thin" and "thick filaments" in a muscle cell consist mainly of actin and myosin, respectively.

Several different proteins are responsible for the anchoring of the actin filaments in the Z-disks (e.g. alpha-actinin and desmin) or at the end of the myofibers in the cell membrane.

Troponin I, -C, -T and Tropomyosin - associated with actin - confer the Ca++-dependent triggering of contraction.

Length of the sarcomere is controlled by the giant protein titin.

In smooth muscle, there is no troponin. Contraction activity is controlled by phosphorylation / dephosphorylation of myosin by a specialized kinase instead. Contractile fibers are not organized in sarcomeres.

Apart from contributing to muscle contraction, the actomyosin system is responsible for many other motions at cellular level, e.g. the amoeboid movement of pseudopodia or the fission of cells at the end of mitosis by a contractile ring.

Besides this, actin fibers fulfill structural tasks like maintenance of the shape of stereocilia or microvilli. Here, actin filaments are connected by proteins like fimbrin. But not

only specialized structures like the mentioned ones contain actin fibers. There is a network covering the complete cell volume with F-actin as a major constituent. Whereas the actin filaments in the structures mentioned above are relatively stable, this F-actin is highly dynamic. Management of the network structure and turnover is achieved by connecting proteins like alpha-actinin, fimbrin or fill-in; turnover is regulated by gelsolin, villin, and different capping- and fragmentation-proteins.

Microtubules are built up of alpha-beta tubulin heterodimers. Turnover of filaments is achieved by building-in and releasing of monomers with different time constant rates at both ends. The resulting cycle is called "treadmilling". Thirteen strings of tubulin duplets build up one subfiber, whereas one fiber contains two or three of those. A complete axoneme consists of 9 radial and 2 central fibers. This "9+2" - structure is the basis both of flagella, their basal bodies and centrioles. In flagella, several additional structures like radial elements exist. Nexin connects the fibers and dyneine is the motor ATPase which shifts the fibers relative to each other. Several genetic diseases like the Cartageneric syndrome are caused by deficiencies of distinct proteins in cilia.

Besides this, microtubules are abundant in all types of cells. They are part of a delivery system for organelles, e.g. in the golgi apparatus. A further very important system based on microtubules is the mitotic spindle, it is organized by the centrosomes. Besides many other components, the major part of a centrosome are two centrioles which are built up of nine microtubule-triplets. Most remarkably, new centrioles are not synthesized de novo but generated by duplication of old ones.

Cytoplasmic microtubules are associated with many different proteins. Two major classes are known: The MAPs ("microtubule-associated proteins", with molecular masses between 200 and 300 kD) and the much smaller tau-Proteins with a MW between 60 and 70 kD. These proteins regulate the treadmill-process and the interaction with other structures in the cell.

Besides actin and myosin the so-called intermediate filaments constitute a third class of filaments. In contrast to the former two groups, they do not participate in motility, nor are they dynamic structures subject to a vivid turnover. The most important ones are

neurofilaments (in neurons), keratin filaments (mainly in epithelial cells), and vimentin filaments (in many sorts different cell types).

The biological function of both the cytoskeleton as well as contractile apparatus of a cell does not end at the cell membrane. Cells must be embedded in the extracellular matrix, all cells of a muscle must act as one single mechanical unit and epithelia must resist macroscopic mechanical forces. Hence, cell adhesion and the extracellular matrix are closely connected to the cytoskeleton. Vincullin is one of the proteins which serve as an anchor for intracellular fibers (actin). Different types of desmosomes and tight junctions connect neighbor cells with intercellular fibers. On the inside, cytoplasmic plaques connect them to the cytoskeleton. These structures, on the one hand, serve as mechanical elements whereas gap junctions, on the other hand, connect cells metabolically.

The extracellular matrix consists of a network of proteins, glycoproteins and polysaccharides. Different proteins are present in relation to different mechanical demands:. Elastin is found in tissues with high elasticity (lungs, heart) whereas collagen, a more hard-wearing protein, is found in tendons and ligaments. Fibronectin is an extracellular protein highly important for cell adhesion.

Reference: Murray J *et al* (1992): Cell Motil Cytoskeleton 22: 211-223.

Within the overall group of Cell Structure and Motility several categories of proteins are coded for by clones of the invention:

Collagen alpha chain proteins: Proteins with the typical (xxG)n repeat of collagen proteins and Pfam von Willebrand factor type A domain(s) suggest they are collagen alpha chains. These proteins can find application in modulation of connective tissue, bone and cartilage development and maintainance. OMIN reports collagen alpha chains have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) Osteogenesis imperfecta, type I (OMIN #166200); 2) Osteogenesis imperfecta congenita (OMIN #166210); 3) Alport Syndrome, X-linked (OMIN #301050); 4) Thrombastenia of Glanzmann and Naegeli (OMIN *273800); 5) Ehlers-Danlos Syndrome, Type VII (OMIN #130060); 6) Marfan Syndrome (OMIN #154700); 7) Alport Syndrome, Autosomal Recessive (OMIN #203780); 8) Alpha-2-Deficient Collagen Disease (OMIN 203760); 9) Goodpasture Syndrome (Omin 233450); 10) Osteogenesis Imperfecta,

progressively deforming, with normal sclerae (OMIN #259420); 11)) Ehlers-Danlos Syndrome, Type VII Autosomal Recessive (OMIN *225410); and 12)) Osteogenesis imperfecta, Type IV (OMIN #166220). OMIN reports that von Willebrand factor type A domains have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases:: 1) Hemophilia A (OMIN *306700); 2) Von Willebrand Disease (OMIN *193400); 3) Giant Platelet Syndrome (OMIN *231200); 4) Thrombastenia of Glanzmann and Naegeli (OMIN *273800); 5) Congenital Thrombotic Diseasae due to protein C deficiency (OMIN #176860); 6) Polycystic Kidney Disease 1 (OMIN *601313); 7) Nephrogenic Diabetes Insipidus (OMIN *304800); 8) Factor V Deficiency (OMIN *227400); and 9) Dentatorubral-Pallidoluysian Atrophy (Omin *125370). Clones in this category include: fbr2_2b5.

Radial spokehead protein: Radial spokehead proteins, e.g., Chlamydomonas reinhardtii radial spokehead protein of flagella or axoneme and the Strongylocentrotus purpuratus sea urchin spermatozoa protein p63, and human proteins with similarity thereto are important for the maintenance of a planar form of sperm flagellar beating. The human protein(s) can find application in modulating the structure of the human spermatozoa radial spoke head and modulation of sperm motility in men (e.g., in sterility). Clones in this category include: tes3_15i5.

Ankyrins: Ankyrins are peripheral membrane proteins which interconnect integral proteins with the spectrin-based membrane skeleton. Thus these proteins are involved in coupling of cyto skeleton and cell membrane. OMIN reports that Ankyrins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) Heriditary Spherocytosis (OMIN *182900); 2) Hemolytic Poikilocytic Anemia due to reduced ankyrin binding sites (OMIN 141700); 3) Atypical Elliptocytosis (OMIN 225450); 4) Autosomal recessive spherocystosis (OMIN #270970); 5) Werner Syndrome (OMIN *277700); and 6) Rhesus-unlinked type Elliptocytosis (OMIN #130600). Clones in this category include: tes3_18l7.

FGD1-related F-actin binding protein (Farbin/FGD1): FGD1-related F-actin-binding protein (Farbin/FGD1) is a novel F-actin-binding protein. The gene locus fgd1 seems to be responsible for faciogenital dysplasia or Aarskog-Scott syndrome. (OMIN 305400). Frabin binds F-actin and shows F-actin-cross-linking activity. Overexpression of frabin in Swiss 3T3 cells and COS7 cells induces cell shape change and c-Jun N-terminal kinase activation, as

described for FGD1. Because FGD1 has been shown to serve as a GDP/GTP exchange protein for Cdc42 small G protein, it is likely that frabin is a direct linker between Cdc42 and the actin cytoskeleton. Cdc42p is an esin yeast, Cdc42p transduces signals to the actin cytoskeleton to initiate and maintain polarized growth and to mitogen-activated protein morphogenesis. In mammalian cells, Cdc42p regulates a variety of actin-dependent events and induces the JNK/SAPK protein kinase cascade, which leads to the activation of transcription factors within the nucleus. Clones in this category include: tes3_72k15.

Paramyosins: Paramyosin is a major structural component of thick filaments and invertebrate muscle. Paramyosins are promising antigens for immunization against several parasites, such as Schistosoma mansoni. Clones in this category include: tes3_7b22.

Tuftelin: Tuftelin/enamelin are matrix proteins of the teeth. As other proteins involved in calcification, these proteins are also expressed in the uterus matrix. The new protein can find application in modulation of tissue-calcification, especially the uterus. As reported by OMIN, tuftelin has been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with amelogenesis imperfecta (OMIN *600087). Clones in this category include: utel_19g22.

Cell Adhesion Regulator (CAR1): CAR1 is involved in the regulation of cell-cell adhesion. OMIN reports the association (as potentially diagnostic, therapeutic, causative, and/or related, etc...) of CAR1 with tumor suppression by the reduction of tumor invasion (OMIN *116935). Clones in this category include: utel_24j6.

Differentiation/Development

Almost every multicellular organism originates from meiotic cell divisions and the recombination of a paternal and a maternal set of chromosomes. After fertilization of the egg, all cells of a body originate from this one cell. Thus the cells of the developing body are initially genetically alike. But phenotypically they become very different. They are specialized to a certain cell type and arranged in an organized pattern to a certain type of tissue and the whole structure has the well-defined shape of an organ. All these features are determined by the DNA sequence of the genome, which is reproduced in every cell. Each cell acts on the genetic instructions given to a certain time and at a certain place of development and plays its individual part in the multicellular organism. Cell differentiation may be divided into three general steps: cell cycle exit, apoptosis protection and tissue specific gene

expression. These processes are coordinated to provide the final and unique tissue characteristics.

An animal cell that has achieved a certain level of development is said to be determined. This differentiation of a cell may be irreversible and in that case the cell may be renewed only by simple duplication. Other cells are renewed by means of stem cells which are immortal (e.g. stem cells of the bone marrow, epidermal stem cells). The genetic control of development is extensively studied in non-vertebrates and vertebrates. The classical animal model is the fruit fly Drosophilia and the modern model is the transgenic mouse. Animal transgenesis has proven to be useful for physiological as well as physiopathological studies. Besides the approach based on the random integration of a DNA construct in the mouse genome, gene targeting can be achieved using totipotent embryonic stem cells for targeted transgenesis. Transgenic mice are than derived from the embryonic stem cells. This allows the introduction of null mutations in the genome (so-called knock-out) or the control of the transgene expression by the endogeneous regulatory sequence of the gene of interest (so-called knock-in). Mice can be created that express wild-type genes, mutant genes, marker genes or cell lethal genes in a tissue specific manner. These animal models allow to follow changes in tissue and organ development and lead to a better understanding of the cellular function of many genes or to the generation of animal models for human diseases. Fundamental problems in immunology, onset and development of cancer, regulation in fatty acid metabolism, aspects of cardiovascular function, control of the central nervous system development, analysis of reproductive development and function are only some examples of research interests.

The final stage of cell differentiation is growth arrest. In animal tissues with rapid cell turnover terminally differentiated cells undergo programmed cell death. The cells have the ability to kill themselves by activating an intrinsic cell suicide program when they are no longer needed or have become seriously damaged. The execution of this program is termed apoptosis. Apoptosis is of importance for development and homeostasis of animals. The key components of this program have been conserved in evolution from worms (C. elegans) to insects (Drosophilia) to humans. The roles of apoptosis include the sculpting of structures during development, deletion of unneeded cells and tissues, regulation of growth and cell number, and the elimination of abnormal and potentially dangerous cells. In this way

apoptosis provides "quality control mechanism" that limits the accumulation of harmful cells, such as virus-infected cells and tumor cells. On the other hand inappropriate apoptosis is associated with a wide variety of diseases, including AIDS, neuro-degenerative disorders and ischemic stroke. Because it is now clear that apoptosis is a result of an active, gene-directed process, it should be eventually possible to manipulate this form of cell death by developing drugs that interact with its recently identified mechanisms of action. Inducers of cell differentiation, cell cycle arrest and apoptosis might be the novel molecular targets for new anticancer agents in addition to the signaling pathways for growth factors and cytokines.

Proteins, factors, receptors and genes of importance in apoptosis:

Proteases:

- Calpain, an intracellular cysteine protease, exact role unknown.

- Caspase-1 to Caspase-11, a family of proteases synthesized as an inactive proenzyme. Targets of the activated enzymes include: poly(ADP-ribose) polymerase, DNA-dependent protein kinase, U1 ribonucleoprotein, nuclear laminins and cytoskeleton components (actin).

- Granzyme B, a serine protease released by cytotoxic T-cells.

Receptors:

- CD 95 (synonyms: Fas, APO-1), a receptor protein of the TNF-receptor family which includes TNF-R1 and TNF-R2 with the common characteristic of a 70 amino acid cytoplasmic domain.

- FADD (synonym: MORT-1), a cytoplasmic protein

- DR-3 (synonym: APO-3) a member of the TNF-receptor-family

- DR-4 and DR-5

Genes:

- ced-3, ced-4 and ced-9 encode the general apoptotic and antiapoptotic program in Caenorhabditis elegans. Apaf-3 is the mammalian homologue of ced-3.

- Bcl-2 / Bcl-xL / Bax / Bcl-xS / Bak: a large gene family that can either inhibit or promote apoptosis.

- Cytokine response modifier A, a cowpox virus gene whose gene product inhibits caspases.

Others:

- Caspase-activated DNase (CAD) and its inhibitor (ICAD), causes DNA fragmentation in the nucleus

- Ceramide, a complex lipid that acts as a second messenger.

- c-Jun N-terminal kinase (JNK) is a proline-directed kinase

- p53 protein, is essential for the induction of apoptosis as a response to chromosomal damage.

- RAIDD, a death signal-transducing protein.

- Receptor interacting protein (RIP) is an accessory protein with a death domain and a serine/threonine kinase activity.

- Sphingomyelinase, an enzyme that hydrolyzes the complex lipid sphingomyelin to ceramide.

- Tumor necrosis factor (TNF) is a type -II membrane protein

- TNF-receptor associated factor (TRAF2), is an accessory protein that can bind to both TNF-R1 and TNF-R2.

Within the overall group of Differentiation/Development, several categories of proteins are coded for by clones of the invention:

Interleukins (e.g. Interleukin-7): Interleukin precursors related to interleukin-7, for example, are expected to act as new growth factors for human B lineage cells. Additionally,

these proteins should induce the gene rearrangement of the T-cell receptor repertoire, leading to thymocyte commitment, and subsequently induce both cytotoxic T-cell- and lymphocyte-activated killer cells These interleukins could find clinical application in a variety of conditions of hematolymphopoietic failure and different tumours, because of its recruitment of B cell lineage cells, cytotoxic T-cell- and lymphocyte-activated killer cells. (OMIN *146660). Clones in this category include: tes3_35e21.

Testis-specific Y-encoded proteins: The TSPY genes are arranged in clusters on the Y chromosome of many mammalian species. TSPY is believed to function in early spermatogenesis and is a candidate for GBY, the putative gonadoblastoma-inducing gene on the Y. Proteins of the TSPY-SET-NAP1L1 family represent proteins closely related to TSPY. These proteins seem to be involved in early spermatogenesis. Clones in this category include: fbr2_2d15.

Intracellular transport and trafficking

Eukaryotic cells rely for their viability on the partitioning of many basic cellular processes into membrane-bounded organelles. These are the nucleus, endoplasmic reticulum (ER), Golgi apparatus, endosomes, lysosomal compartments, mitochondria and peroxisomes. Most molecules destined for the lysosome, cell surface and outside the cell are routed through the ER and Golgi, which together with the vesicular intermediates between them, comprise the secretory pathway (Palade 1975). In the ER and Golgi compartments proteins are sorted, modified and often assembled into complexes *en route* to their final destination. Incorrectly assembled proteins are retained in the ER until they fold correctly or are targeted for degradation. Additional proteins are translocated into and function within the lumenal spaces of organelles or are secreted. Thus a large proportion of proteins synthesized require targeting to membranes either for insertion into or transport across them. A major purpose of this is growth. The secretory pathway is dependent on an intact cytoskeleton and also closely linked to general metabolism by affecting ribosome biogenesis (Mizuta and Warner, 1994). A huge number of proteins is required for targeting, translocation and sorting of newly synthesized proteins.

The first step in sorting is the recognition of cis-acting targeting or signal sequences that organelle-targeted proteins contain. This is carried out by cytosolic targeting factors and/or receptors on the membrane to which the protein is targeted. In some cases the primary

sequences are extremely degenerate, with only the overall character being conserved (hydrophobicity for an ER signal sequence, helical amphiphilicity for mitochondrial targeting sequence (Kaiser *et al.*, 1987; Lemire *et al.*, 1989). Following the targeting step, proteins are either inserted into or transported across the membrane (translocated) through a proteinaceous apparatus (termed the translocon). The translocon include or recruit motors to drive the translocation process in the correct direction (Schatz and Dobberstein, 1996).

Defined intracellular protein transport steps:

- ER
  - targeting to the ER
  - translocation into the lumen of the ER, and, depending on the presence of certain signals in the peptide sequence transport through the golgi complex
- Mitochondria
  - targeting
  - translocation
- Peroxisomes
- The general secretory pathway
  - protein modification, assembly and quality control in the ER
  - vesicle-mediated trafficking
  - vesicle docking and fusion
  - transport through the golgi apparatus and sorting at the trans-golgi
  - transport to the cell surface
  - transport routes to the lysosome
- Endocytosis
- Specialized protein transport routes
- Protein export from the cytoplasm

References: Palade, G (1975) Science 189:347-358; Mizuta et al. (1994) Mol Cell Biol 14: 2493-2502; Kaiser *et al.* (1987) Science 235: 312-317; Lemire *et al.* (1989) J Biol Chem 264: 20206-20215; Schatz et al. (1996) Science 271: 1519-1526.

Rab proteins

In eukaryotic cells the compartmentalisation of processes is a prerequisite for a tight regulation of processes and activities. The cells contain a highly dynamic set of membrane compartments that are responsible for packaging, sorting, secreting, and recycling proteins

and other molecules. Trafficking between organelles within the secretory pathway occurs as vesicles derived from a donor compartment fuse with specific acceptor membranes, resulting in the directional transfer of cargo molecules. This process is tightly controlled by the Rab/Ypt family of proteins (reviewed by Novick and Zerial, 1997), a branch of the superfamily of small GTPases. Rab proteins regulate a variety of functions, including vesicle translocation and docking at specific fusion sites. Rabs may also play critical roles in higher order processes such as modulating the levels of neurotransmitter release in neurons, a likely mechanism in synaptic plasticity that underlies learning and memory (Geppert and Südhof, 1998).

Small GTPases share a common three-dimensional fold that, in the GTP bound state, can bind a variety of downstream effector proteins. GTP hydrolysis leads to a conformational change in the "switch" regions that renders the GTPase unrecognizable to its effectors. In this way, by localizing and activating a select set of effectors, a common structural motif is used to control a wide array of distinct cellular processes.

The final steps in membrane fusion are likely to be driven by a set of proteins known as SNAREs. After a vesicle becomes docked, the cytoplasmic domains of VAMP (also termed synaptobrevin) and syntaxin on opposing membranes, in combination with a SNAP-25 molecule, coalesce into an elongated -helical bundle (Poirier et al., 1998 ; Sutton et al., 1998), which may lead to fusion. Because numerous SNARE isoforms have been identified that localize to distinct membrane compartments, it was originally proposed that the specificity of interaction between the SNARE proteins accounted for the specificity in membrane trafficking. Recent results, however, suggest that SNAREs are not specific in their ability to form complexes in vitro, suggesting that trafficking specificity requires additional factors (Yang et al., 1999). In this regard, Rab proteins are strong candidates for governing the specificity of vesicle trafficking. Like the SNAREs, many isoforms (40) of the Rab family have been identified that localize to specific membrane compartments (reviewed by Novick and Zerial, 1997).

Concomitant with the SNARE cycle, Rab proteins undergo a intricate cycle of membrane and protein interactions. Rabs are posttranslationally modified at C-terminal cysteines by the addition of two geranylgeranyl groups, which mediate membrane association when the Rab is in the GTP-bound state. After guanine nucleotide hydrolysis occurs, the Rab is extracted from the membrane upon forming a complex with a cytosolic GDP-dissociation

inhibitor (GDI). This cytosolic intermediate is then recycled onto a newly forming vesicle, most likely through a secondary factor termed a GDI dissociation factor (GDF), which displaces GDI. After the Rab becomes membrane bound, a guanidine nucleotide exchange factor (GEF) promotes release of GDP and the subsequent loading of GTP. In its GTP-bound conformation, the Rab is then free to associate with its specific set of effectors, which can in turn trigger events leading to the eventual fusion of the vesicle with a target membrane. To complete the cycle, perhaps after or concurrent with membrane fusion, a GTPase activating protein (GAP) accelerates nucleotide hydrolysis, switching off the GTPase. The remaining GDP-bound Rab can then participate in a new round of fusion.

Rab interactions with effectors are likely to regulate vesicle targeting and membrane fusion in three ways. First, a Rab may specifically facilitate vectorial vesicle transport. Vesicles are transported from their site of origin to acceptor compartments likely through associations with cytoskeletal elements and transport motors. A protein has been identified with a domain structure that suggests a connection between the cytoskeleton and the Rabs. This protein, called Rabkinesin-6, contains a kinesin-like ATPase motor domain followed by a coiled-coil stalk region and a RBD that specifically binds Rab6 (Echard et al., 1998). An additional link with the cytoskeleton is provided by the Rab effector, Rabphilin-3A. Rabphilin-3A has been shown in vitro to interact with -actinin, an actin-bundling protein, but only when not bound to Rab3A (Kato et al., 1996). These results raise the intriguing possibility that Rab proteins regulate vesicle interactions with the cytoskeleton and thereby play an active role in targeting vesicles to their appropriate destinations.

Second, Rab proteins may regulate membrane trafficking at the vesicle docking step. A number of Rab effectors, including Rabaptin-5, EEA1, Rabphilin-3A, and Rim, may serve as molecular tethers. Each effector protein contains a RBD, followed by a linker region (some having the potential to form elongated coiled-coil structures), and a domain capable of interacting with a second Rab or the target membrane. Rabaptin-5, for example, contains two RBDs, one near the N terminus that specifically recognizes Rab4 and a second near the C terminus that binds Rab5 (Vitale et al., 1998). Both Rim, which is localized to the target membrane, and Rabphilin-3A, which is localized to the vesicle, contain N-terminal RBDs and C-terminal Ca2+-binding C2 domains, implicating these effectors in synaptic vesicle localization or docking in response to Ca2+ influx (Wang et al., 1997). Tethering effectors may also recognize protein complexes on the acceptor membrane. Sec4p, a yeast Rab3A

homolog, interacts with the exocyst (Guo et al., 1999), a complex of seven or more subunits that is assembled at sites of vesicle fusion along the plasma membrane. The exocyst complex may therefore function as a landmark for Rab/effector-mediated vesicle docking.

Third, once a vesicle has become tethered to its fusion site, Rab proteins may selectively activate the SNARE fusion machinery. The mechanism of this activation is unknown but may involve direct interactions of Rabs or, more likely, their effectors with SNAREs. For example, Hrs-2 is a protein that binds to SNAP-25 and contains a Zn2+-finger motif characteristic of Rab-binding proteins such as Rabphilin-3A, Rim, EEA1, and Noc2, suggesting that Hrs-2 may form a physical link between Rabs and SNAREs (Bean et al., 1997). In addition, certain mutations in the syntaxin-binding protein Sly1p, the Sec1p homolog utilized in ER to Golgi trafficking, eliminate the requirement for Ypt1p, a Rab protein that functions at this trafficking step (Dascher et al., 1991). Rabs may therefore regulate SNARE associations through Sec1 family members. In support of this idea, a Rab effector was recently found to interact with a vacuole Rab, a Sec1p homolog, and a SNARE protein (Peterson et al., 1999), which suggests that this effector serves to connect Rab and SNARE function. In this way, Rabs and their effectors may facilitate the correct pairing of SNAREs.

References: Dascher et al. (1991) Mol. Cell. Biol. 11, 872-885; Echard et al. (1998). Science. 279, 580-585; Geppert et al. (1998) Annu. Rev. Neurosci. 21, 75-95; Guo et al. (1999). EMBO J. 18, 1071-1080; Kato et al. (1996) J. Biol. Chem. 271, 31775-31778; Novick et al. (1997) Curr. Opin. Cell Biol. 9, 496-504; Peterson (1999) Curr. Biol. 9, 159-162; Poirier et al. (1998) Nat. Struct. Biol. 5, 765-769; Vitale et al. (1998) EMBO J. 17, 1941-1951; Wang et al. (1997) Nature. 388, 593-598; Yang et al. (1999) J. Biol. Chem. 274, 5649-5653.

Within the overall group of Intracellular Transport and Trafficking several categories of proteins are coded for by clones of the invention.

Rab proteins:

Rab1B is essential for the intracellular transport of nascent low density lipoprotein (LDL) receptor. It is discussed as a universal mediator of endoplasmatic reticulum to Golgi transport of membrane glycoproteins in mammalian cells. . Clones in this category include: fbr2_2i17, fbr2_3b16.

Rab10 appear concentrated on membranes in the perinuclear region. Rab 10 has been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases as reported by OMIN: 1) Choroideremia (OMIN *303199); and 2)RETT Syndrome (OMIN 312750). Clones in this category include: fbr2_62l19.

In mice, Rab17 shows epithelial cell specificity. Rab 17 is discussed as candidate gene for the mouse mutations ln (leaden), Tw (twirler), and ax (ataxia). Cloned from a brain cDNA library, the new putative Rab-protein is expected to be involved in vesicle trafficking within neuronal cells. These proteins can find application in modulating the transport of vesicles inside neuronal cells, which are essential for development of functional dendritic processes. . . Clones in this category include: fbr2_41m15.

Ankyrin G: The ankyrin 3 gene encodes a novel ankyrin, which is expressed in multiple tissues, with very high expression at the axonal initial segment and nodes of Ranvier of neurons in the central and peripheral nervous systems. Ankyrin G shows several tissue-specific alternative mRNA processing. The different ankyrin G proteins participate in maintenance/targeting of ion channels and cell adhesion molecules to nodes of Ranvier and axonal initial segments. Ankyrin G has been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with Werner disease (OMIN *277700). Clones in this category include: fkd2_24p5.

Zn-T-transporters: The Zn-T-transporters are membrane proteins that facilitates sequestration of zinc in endosomal vesicles. In the brain, ZnT-3 mRNA seems to be involved in the accumulation of zinc in synaptic vesicles. Zinc (Zn) is an essential element in normal development and metabolism. Recent studies show that in Alzheimer's disease, Zn functions as a double-edged sword, affording protection against Alzheimer's amyloid beta peptide (the major component of senile plaques) at low concentrations and enhancing toxicity at high concentrations by accelerated aggregation of the amyloid beta peptide. These proteins can find application in modulation of Zinc transport in neuronal cells, thus providing means for a modulation of Alzheimer's amyloid beta peptide plaque formation. (OMIN *602878, *602095). Clones in this category include: fbr2_62f10.

Metabolism

This group includes proteins which are involved in the uptake and consumption of nutrients, and enzymes which are part of the biochemical pathways for energy metabolism or

which are involved in the supply of building blocks of nucleic acids, proteins (NTPs, dNTPs, amino acids) for DNA/RNA and protein synthesis, and fatty acids (membranes), to allow for the generation of higher order structures. This group constitutes the most important and largest group in prokaryotes and lower eukaryotes. The higher the evolutionary level of an organism is, however, the more other protein classes like 'signal transduction', 'cell cycle' and 'differentiation and development' increase in importance and number of representatives.

Proteins involved in the metabolism of energy and compounds (here: other than nucleic acids or proteins) are usually the products of house keeping genes, they are often constitutively and/or ubiquitously expressed.

Several categories of proteins are coded for by clones of the invention within the overall group of Metabolism:

NAT1, ARD1: In yeast, ARD1 and NAT1, are required for the expression of an N-terminal protein acetyltransferase 1. NAT1 controls full repression of the silent mating type locus HML, sporulation and entry into G0. ARD1 is involved in the assembly of the NAT 1-complex. These can find application modulating NAT assembly and action and therefore could be important in metabolism of drugs and environmental mutagens.(OMIN *108345). Clones in this category include: fbr2_3g8.

Apolipoprotein E receptor: In LDL-receptors the class A domains form the binding site for LDL and calcium. The acidic residues between the fourth and sixth cysteines are important for high-affinity binding of positively charged sequences in LDLR's ligands. These proteins can find application in modulation of cholesterol binding and transport by LDL-receptors and LDL-binding proteins. In normal individuals, chylomicron remnants and very low density lipoprotein (VLDL) remnants are rapidly removed from the circulation by receptor-mediated endocytosis in the liver. In familial dysbetalipoproteinemia, or type III hyperlipoproteinemia (HLP III), increased plasma cholesterol and triglycerides are the consequence of impaired clearance of chylomicron and VLDL remnants because of a defect in apolipoprotein E. Accumulation of the remnants can result in xanthomatosis and premature coronary and/or peripheral vascular disease. OMIN reports that apolipoprotein has associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) Familial hypercholesterolemia (OMIN 143890); 2) Familial combined hyperlipidemia (OMIN 144250); and 3) Alzheimer disease. (OMIN #104300). Clones in this category include: fbr2_62017.

Ubiquitin carboxyl-terminal hydrolases: Ubiquitin carboxyl-terminal hydrolases (EC 3.1.2.15) (UCH) (deubiquitinating enzymes) are thiol proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquinated proteins. OMIN reports that Ubiquitin-specific proteases have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) Lung carcinoma (OMIN *603486); 2) x-linked retinal diseases (OMIN *300050); 3) oncogenesis (OMIN *300050);4) ovarian cancer (OMIN *300050). Clones in this category include: fbr2_78k24; htes3_27d1.

Phosphoserine signature (phosphoglucomutases, phosphomannomutase): These proteins take part in the conversion of hexose phosphates. OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following disease: Fanconi-Bickel Syndrome (OMIN #227810). Clones in this category include: fkd2_24b15.

NADH ubiquinone oxidoreductase: NADH:ubiquinone oxidoreductase is the first enzyme in the respiratory electron transport chain of mitochondria. It is a a membrane-bound multi-subunit protein. The bovine heart enzyme contains about 40 different polypeptides. OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following disease: Brancio-oto-renal syndrome (OMIN *6601445). Clones in this category include: fkd2_3o17.

Transketolases: Transketolase requires thiamin pyrophosphate as cofactor and shows a wide specificity for both reactants, e.g. converts hydroxypyruvate and R-CHO into CO(2) and R-CHOH-CO-CH(2)OH. OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: Wernicke-Korsakoff Syndrome (OMIN *277730). Clones in this category include: tes3_17l17.

Fatty acid-CoA synthetases/ligases: These proteins contain AMP-binding domain signature(s), which is present in enzymes which act via an ATP-dependent covalent binding of AMP to their substrate. This domain is found in several CoA synthetases, such as acetate-CoA ligase (EC 6.2.1.1), long-chain-fatty-acid-CoA ligase (EC 6.2.1.3), bile acid-CoA ligase. OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic,

causative, and/or related, etc...) with the following diseases: 1) Alport syndrome , mental retardation and elliptocytosis (OMIN *300157); 2) Adrenoleukodystrophy (OMIN *300100). Clones in this category include: tes3_35k17.

ADP/ATP or Adenine Nucleotide Translocataors: These proteins contain mitochondrial energy transfer signature(s) and are most abundant in mitochondria. In its functional state, it is a homodimer of 30-kD subunits embedded asymmetrically in the inner mitochondrial membrane. The dimer forms a gated pore through which ADP is moved from the matrix into the cytoplasm.. OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) cardiomyopathy (OMIN *103220); 2) myopathy (OMIN *103220); 3)Progressive external ophthalmoplegia (OMIN *601227). Clones in this category include: tes3_35n12.

Carboxylesterases: OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1)hepatic carboxylesterase with detoxification of foreign compounds (OMIN *114835); 2) non-Hodgkin lymphoma (OMIN *114835); 3) B-cell chronic lymphocytic leukemia (OMIN *114835); 4) rheumatoid arthritis (OMIN *114835). Clones in this category include: tes3_35n9.

Heat shock proteins: OMIN reports that these proteins have associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1)27 kd heat shock protein has been correlated with thermotolerance in response to environmental challenges and developmental transitions. (OMIN *6021295). Clones in this category include: utel1_23e13.

Nucleic acid management

The genetic information is stored in the form of nucleic acids in all organisms. Two kinds of nucleic acids exist, DNA and RNA. Whereas the more stable DNA in most organisms constitutes the storage form of the genetic information, the labile RNA and in particular mRNA is an intermediate used for the temporal expression of specific genes.

In eukaryotes, DNA is usually a double stranded linear molecule consisting of two antiparallel strands and made up of a deoxyribose, a phosphorus backbone and the four bases A, C, G, and T. The DNA of some organisms has a ring structure. The structure of DNA was

unraveled years ago by Watson and Crick. DNA is directional molecule determined by the C-atoms of the sugar.

The most important processes dealing with nucleic acids are:

- replication (e.g. DNA polymerases, Telomerase)
- transcription (RNA polymerases)
- RNA processing (maturation - splicing and degradation)
- in addition, enzymes and proteins exist which require a nucleic acid (mostly RNA) in the active center to be functional (ribozymes - e.g. RNase, Ribosomal proteins)

The DNA of a cell is replicated in the S-phase of the cell cycle. Several enzymes carry out the task of doubling this nucleic acid. As all steps of the cell cycle, also the process of replication is tightly regulated. The enzyme DNA polymerase and several other proteins are involved in this process. Whereas many prokaryotes do have only one origin of replication (i.e., the starting point of the replication cycle), in eukaryotic DNAs (chromosomes) multiple such start points exist. The switch from the synthesis (S) phase to the subsequent G2 or M phases of the cell cycle are dependent on the completion of the replication. This makes clear, that a number of proteins are involved in the replication itself as well as in the control of the process. Since most eukaryotic chromosomes are linear structures, additional proteins and enzymes are necessary to make sure that the structure is maintained through successive generations. This includes those proteins necessary to build the three dimensional structure of chromosomes (e.g. histones) and the structural network of the nucleus and nucleolus (including the defined localization of transcriptionally active genes in the vicinity of nucleoli) but also such enzymes as telomerase which guarantees the integrity of the chromosomal ends.

The expression of genes is usually performed in two steps. First a messenger RNA (mRNA) is produced (transcribed) in one to many copies and second this mRNA is translated into the protein product. The regulation of transcription is discussed under the separate heading 'transcription factors', but also the classes 'signal transduction', 'development', 'cell cycle' and others are affected as the expression of certain genes determines the fate of a cell or organism.

The primary transcript (hnRNA - heterogeneous nuclear RNA) is a single stranded one-to-one copy of the gene as it is located on the chromosome. Before a protein can be translated, already during transcription the process of maturation is initiated. Firstly, a 5' cap structure is enzymatically and covalently added to the RNA, blocking the 5' end of the RNA.

Second, when the RNA polymerase has terminated polymerization, the enzyme poly A polymerase adds varying numbers of adenine residues to the 3' end of the transcript. This enzyme recognizes the sequence AAUAAA or AUUAAA (+ some minor variations), cuts the RNA 10 - 30 nucleotides downstream and adds the A residues. The size of the poly A sequence affects the stability of the RNA. Finally, in the process of splicing, the introns present on the genomic level and also present in the hnRNA are spliced out by a multi-protein complex consisting of several proteins and RNAs. The finally maturated mRNA is exported to the cytoplasm where it is translated with help of the ribozymes.

The half life of RNA is usually much shorter than that of DNA. Usually, the mRNA is degraded shortly after synthesis, to guarantee a very defined window of expression of a given gene. This regulation is necessary to specifically maintain or change the set of proteins present at any time in a cell. Specific regions in the 3'UTR (untranslated region) determine the stability of the mRNA in the cytoplasm before it is degraded by RNases, enzymes consisting both of protein and RNA.

References: Watson and Crick (1953) Nature **171**: 737-738.

Several categories of proteins are coded for by clones of the invention within the overall group of "Nucleic acid management"and include, among others, the following:

RNA helicases including DEAD/H box helicases: RNA helicases comprise a large family of proteins that are involved in basic biological systems such as nuclear and mitochondrial splicing processes, RNA editing, rRNA processing, translation initiation, nuclear mRNA export, and mRNA degradation. RNA helicases are essential factors in cell development and differentiation, and some of them play a role in transcription and replication of viral single-stranded RNA genomes. The members of the largest subgroup, the DEAD and DEAH box proteins, exhibit a strong dependence of the unwinding activity on ATP hydrolysis. DEAD box proteins have been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) as reported by with the following disease processes and/or genes: 1) ataxia-telangiectasia gene: "A human gene (DDX10) encoding a putative DEAD-box RNA helicase at 11q22-q23" *Genomics* 33:199-206, 1996, Savitsky et al., (OMIN *601235); 2) hematopoetic tumors: "Cloning and expression of a murine cDNA homologous to the human RCK/P54, a lymphoma-linked chromosomal breakpoint 11q23", Gene 166:293-6, 1995, Seto et al. (OMIN *600326); 3) dermatomyositis: a) "The major dermatomyositis-specific Mi-2 autoantigen is a presumed helicase involved in transcriptional activation."

Arthritis Rheum. 38: 1389-1399, 1995, Seelig et al. (OMIN *603277); b) "Two forms of the major antigenic protein of the dermatomyositis-specific Mi-2 autoantigen." (Letter), *Arthritis Rheum.* 39: 1769-1771, 1996., Seelig et al. (OMIN *603277); c) "The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities", *Cell* 95: 279-289, 1998. Zhang et al. (OMIN *603277); 4) Muscular Dystrophy, Pseudohypertrophic Progressive Duchenne and Becker Types (OMIN *310200); 5) Mucopolysaccharidosis Type IVA (OMIN *253000); 6) Albinism I (OMIN *203100); 7) Wilms Tumor 1 (OMIN *194070); 8) Spinocerebellar Ataxia 7 (OMIN *164500). Clones in this category include: fbr2_23b10, fbr2_3c18, fbr2_6o17, fbr2_82i24, and tes3_14h21.

Inorganic pyrophosphatase: Inorganic pyrophosphatase (EC 3.6.1.1) (PPase) is the enzyme responsible for the hydrolysis of pyrophosphate (PPi) which is formed as the product of the many biosynthetic reactions that utilize ATP. All known PPases require the presence of divalent metal cations, with magnesium conferring the highest activity. Clones in this category include: fbr2_64a15.

DNA-damage –inducible protein (dinP) or Proteins induced by DNA-Damage: The dinB/P pathway is a second SOS-pathway in E.coli. Genes related to this seem to be involved in modulating DNA repair and mutagenesis. Clones in this category include: fbr2_72b18.

Proteins with myc-type, helix-loop-helix dimerization domain signature(s). This helix-loop-helix domain mediates protein dimerization has been found in proteins such as the myc family of cellular oncogenes, proteins involved in myogenesis and vertebrate proteins that bind specific DNA sequences in various immunoglobulin chains enhancers. Therefore, these proteins could be novel DNA-binding proteins. Clones in this category include: fbr2_72l12.

Cytosolic ribosomal proteins L36: L36 seems to be part of the eukaryotic ribosomal peptidyl transferase center and can find application in modulation of ribosome assembly, maintenance and activity. Clones in this category include: fkd2_3b2.

Ribonuclease H: Ribonuclease H proteins are RNA modificating proteins and have been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases as reported by OMIN: 1) Adenomatous Polyposis of the Colon (OMIN

*175100); 2) Retinoblastoma (OMIN *180200) ; and 3) Von Hippel-Lindau Syndrome (OMIN *193300). Clones in this category include: phtes3_15j3.

Signal transduction

Cells in higher order organisms need to continuously communicate with its environment especially with other cells of the same organism in order to maintain the function and specialization of the whole system these cells are part of. This important task of communication is performed with help of cell-surface receptors which receive and transmit signals from outside into the cell.

G-proteins

The largest known family of cell-surface receptors is that of the G-protein-coupled receptors, which mediate the transmission of diverse stimuli such as neurotransmitters, glycopeptides, hormones, peptides, odorant molecules, and photons. The functional unit of these receptors is composed of the receptor molecule itself (GPCR) which is anchored in the cytoplasma membrane with seven membrane spanning domains, the heterotrimeric G-protein which is composed of α and βγ-subunits (Gα and Gβγ), and the effectors that interact with Gα and / or Gβγ. In particular, the dissociated Gα and Gβγ can regulate the activities of a number of effector molecules such as adenylate cyclases, phopholipase C isoforms, ion channels, and tyrosine kinases, resulting in a variety of cellular functions. The process of signal transduction must be tightly regulated and reversible in order to avoid overstimulation, to achieve signal termination, and render the receptor responsive to subsequent stimuli [Iacovelly L. et al., (1999) *FASEB J.* **13**, 1-8, Hamm, H.E. (1998) *J. Biol. Chem.* **273**, 669-672].

G-proteins are GTPases that, upon binding of GTP change their conformation which in return unmasks structural motives, in particular the so called effector loop, which can mediate the interactions to target proteins, or effectors, for the GTPases. This ability enables the GTPases to cycle between active, GTP-bound and inactive, GDP bound conformations and in the process to function as molecular traffic lights in a multitude of signal transduction pathways. The most important of these signal transduction pathways that are regulated with help of G-proteins are that of the phospholipase C / protein kinase C and that of the adenylate cyclase / protein kinase A.

The cycling of GTPases is tightly regulated by three main classes of proteins: The exchange of hydrolyzed GDP for a fresh GTP is facilitated by guanosine nucleotide exchange factors (GEFs), the hydrolysis of GTP to GDP is sped up by GTPase-activating proteins (GAPs), and the dissociation of GDP from the GTPases is inhibited by GDP dissociation inhibitors (GDIs) [Tapon and Hall (1997) *Curr.Opin. Cell. Biol.* **9**, 86-92, Van Aelst and D-Souza-Schorey (1997) *Genes Dev.* **11**, 2295-2322].

SOC-family

A conserved motif that was originally identified in proteins that negatively regulate the signaling action of cytokines was termed SOCS box, the Suppressor Of Cytokine Signaling. Based on homology, five distinct structural protein classes have been identified since that carry this motif. The function of most of these proteins is presently not known. Common to the proteins is only the SOCS box which is located near the C-terminus of the respective peptides. Recently, the SOCS box has been demonstrated to induce binding of proteins to elongins B and C which could target the proteins (and bound substrates) to the proteasomal protein degradation pathway (Kamura, T. *et al.* (1998) *Genes Dev.* **12**, 3872-3881; Zhang, J.-G. *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**, 2071-2076).

The class where the SOCS box was originally described contains several members (SOCS-1-SOCS-7 and CIS). In addition to the SOCS box, these proteins also contain a SH2 (Src-homology 2) domain and a variable N-terminus. These SOCS proteins appear to form part of a classical negative feedback loop that regulates cytokine signal transduction. Upon cytokine stimulation, expression of SOCS proteins is rapidly induced and the proteins inhibit further cytokine action. The mode of action of the SOCS proteins is variable. While SOCS-1 binds and inhibits the JAK (Janus kinases) family of cytoplasmic protein kinases [Narahzaki M. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**, 13130-13134, Nicholson, S.E. *et al.* (1999) *EMBO. J.* **18**, 375-385], CIS appears to act by competing with signaling molecules such as the STATs (Transducers and Activators of Transcription) family for binding to phosphorylated receptor cytoplasmic domains [Yoshimura, A. *et al.* (1995) *EMBO J.* **14**, 2816-2826; Matsumoto, A. *et al.* (1997) *Blood* **89**, 3148-3154].

A second class of SOCS box protein contains additionally WD-40 repeats which were initially identified in the mouse WSB-1 and -2 proteins. The functions of WD-40 proteins are not completely understood but seem to be rather divergent. In Cdc4p the WD-40 repeats probably are necessary for binding the substrate for Cdc34p [Mathias, N. *et al.* (1999) *Mol.*

Cell Biol. **19**, 1759-1767]. Cdc4p is a component of a ubiquitin ligase that tethers the ubiquitin-conjugating enzyme Cdc34p to its substrates. The posttranslational modification of a protein by ubiquitin usually results in rapid degradation of the ubiquitinated protein by the proteasome. The transfer of ubiquitin to substrate is a multistep process where WD-40 repeats might play an important function.

Other WD-40 containing proteins (e.g. the retino blastoma binding protein RbAp48) have been shown to bind metal ions (Zinc) and that this metal binding might mediate and/or regulate protein-protein interactions which are functionally important in chromatin metabolism [Kenzior, A.L. and Folk, W.R. (1998) *FEBS Lett.* **440**, 425-429]. These proteins are involved in the RAS-cAMP pathway that regulates cellular growth [Ach R.A. *et al.* (1997) *Plant Cell* **9**, 1595-1606].

The SPRY domain has been identified in pyrin or marenostrin, a protein which is mutated in patients with Mediterranean fever and which is similar to the butyrophilin family. While butyrophilins seem to be involved in the lactation process in mammals, the function pyrin is unknown. Three proteins (SSB-1 to -3) have been identified to contain both SPRY and SOCS box motifs. The function of these proteins is also not known.

Ankyrin repeat containing proteins share a 33-residue repeating motif, an L-shaped structure with protruding β-hairpin tips which mediate specific macromolecular interactions with cytoskeletal, membrane, and regulatory proteins. These proteins play fundamental roles in diverse biological activities including growth and development, intracellular protein trafficking, the establishment and maintenance of cellular polarity, cell adhesion signal transduction, and mRNA transcription. Three proteins that contain ankyrin repeats (ASB-1 to -3) have been identified to contain a C-terminal SOCS box additionally to the ankyrin repeats. The function of these proteins or the individual domains remains to be discovered [Hilton, D.J. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**, 114-119].

A few small GTPases (RAR and RAR like) do also contain a SOCS box. GTPases are involved in signal transduction during cellular communication. The function of the SOCS box in this type of proteins is currently unclear [Hilton, D.J. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**, 114-119].

<u>Ca^{2+} as second messenger</u>

The bivalent cation Ca^{2+} is, besides cAMP, one of the two major second messengers in eukaryotic cells. Its intracellular concentration is tightly regulated and usually kept very

low compared to the cell's environment. Ca^{2+} binding proteins and transporters (Gap junction, Voltage-gated, second messenger-gated) help to sequester huge amounts of the ion in various organelles from where Ca^{2+} can be released upon extracellular stimuli. E.g. the contraction of the muscle is dependent on the presence of Ca^{2+} ions which are readily transported back into the organelles in order for the muscle to relax. In signal transduction, Ca^{2+} functions as a second messenger that activates Ca^{2+} dependent processes through the activation of Ca^{2+}/calmodulin dependent protein kinases (CaM kinases) which are the major effector molecules of Ca^{2+}. In the signaling cascades, the CaM dependent kinases activate phospholipases (e.g. phospholipase C) that in return activate other protein kinases such as protein kinase C.

cAMP

The cyclic AMP is produced by the enzyme adenylate cyclase in response to extracellular signals. Certain G-proteins stimulate the activity of adenylate cyclase which converts ATP to cAMP and PPi. Two molecules of cAMP bind to each of two regulatory subunits of cAMP dependent protein kinase which in turn dissociate from the two catalytic subunits of the heterotetramer R_2C_2. Upon release of the C-subunits, they become active and phosphorylate substrate proteins at Ser and Thr residues. The process leading from binding of extracellular molecules to their receptors, the transmission of the stimuli into the cell, the activation of adenylate cyclase and the subsequent activation of cAMP dependent protein kinase is one of two major signal transduction pathways in eukaryotic cells. Since the phosphorylation of proteins is a posttranslational modification of proteins, the kinases are described in the class "signal transduction."

SARA

Members of the transforming growth factor ß (TGFß) superfamily signal through a family of cell-surface transmembrane serine/threonine kinases, known as type I and type II receptors (Heldin et al., 1997 ; Attisano and Wrana, 1998 ; Kretzschmar and Massagué, 1998). Ligand induces formation of heteromeric complexes of these receptors, and signaling is initiated when receptor I is phosphorylated and activated by the constitutively active kinase of receptor II (Wrana et al., 1994). The activated type I receptor kinase then propagates the signal to a family of intracellular signaling mediators known as Smads (contraction of the C.elegans Sma and Drosophila Mad genes which were the first identified members of this class of signaling effectors).

Three classes of Smads with distinct functions have been defined: the receptor-regulated Smads, which include Smad1, 2, 3, 5, and 8; the common mediator Smad, Smad4; and the antagonistic Smads, which include Smad6 and 7 (Heldin et al., 1997; Attisano and Wrana, 1998 ; Kretzschmar and Massagué, 1998). Receptor-regulated Smads (R-Smads) act as direct substrates of specific type I receptors, and the proteins are phosphorylated on the last two serines at the carboxyl terminus within a highly conserved SSXS motif (Macías-Silva et al., 1996 ; Abdollah et al., 1997 ; Kretzschmar et al., 1997 ; Liu et al., 1997b ; Souchelnytskyi et al., 1997). Regulation of R-Smads by the receptor kinase provides an important level of specificity in this system. Thus, Smad2 and Smad3 are substrates of TGFß or activin receptors and mediate signaling by these ligands (Macías-Silva et al., 1996 ; Liu et al., 1997b ; Nakao et al., 1997), whereas Smad1, 5, and 8 are targets of BMP receptors and propagate BMP signals (Hoodless et al., 1996 ; Chen et al., 1997b ; Kretzschmar et al., 1997 ; Nishimura et al., 1998). Once phosphorylated, R-Smads associate with the common Smad, Smad4 (Lagna et al., 1996 ; Zhang et al., 1997), and mediate nuclear translocation of the heteromeric complex. In the nucleus, Smad complexes then activate specific genes through cooperative interactions with DNA and other DNA-binding proteins such as FAST1, FAST2, and Fos/Jun (Chen et al., 1996 , Chen et al., 1997a ; Liu et al., 1997a ; Labbé et al., 1998 ; Zhang et al., 1998 ; Zhou et al., 1998). In contrast to R-Smads and Smad4, the antagonistic Smads, Smad6 and 7, appear to function by blocking ligand-dependent signaling (reviewed in Heldin et al., 1997).

Phosphorylation of R-Smads by the type I receptor is essential for activating the TGFß signaling pathway (Heldin et al., 1997 ; Attisano and Wrana, 1998 ; Kretzschmar and Massagué, 1998). However, little is known of how Smad interaction with receptors is controlled. A novel Smad2/Smad3 interacting protein has been described (Tsukazaki T. et al., 1998) that contains a double zinc finger, or FYVE domain, and which has been called SARA (Smad anchor for receptor activation). The SARA motif recruits Smad2 into distinct subcellular domains and co-localizes and interacts with TGFß receptors. TGFß signaling induces dissociation of Smad2 from SARA with concomitant formation of Smad2/Smad4 complexes and nuclear translocation. Moreover, deletion of the FYVE domain in SARA causes mislocalization of Smad2 and inhibits TGFß-dependent transcriptional responses. Thus, SARA defines a component of TGFß signaling that functions to recruit Smad2 to the receptor by controlling the subcellular localization of Smad.

References: Abdollah et al. (1997) J. Biol. Chem. 272, 27678-27685; Attisano et al. (1998) Curr. Opin. Cell Biol. 10, 188-194; Chen et al. (1996) Nature 383, 691-696; Chen et al. (1997a) Nature 389, 85-89; Chen et al. (1997b) Proc. Natl. Acad. Sci. USA 94, 12938-12943; Heldin et al. (1997) Nature 390, 465-471; Hoodless et al. (1996) Cell 85, 489-500; Kretzschmar et al. (1998) Curr. Opin. Genet. Dev. 8, 103-111; Kretzschmar et al. (1997) Genes Dev. 11, 984-995; Labbé et al. (1998) Mol. Cell 2, 109-120; Lagna et al. (1996) Nature 383, 832-836; Liu et al. (1997a) Genes Dev. 11, 3157-3167; Liu et al. (1997b) Proc. Natl. Acad. Sci. USA 94, 10669-10764; Macías-Silva et al. (1996) Cell 87, 1215-1224; Nakao et al. (1997) EMBO J. 16, 5353-5362; Nishimura et al. (1998) J. Biol. Chem. 273, 1872-1879; Souchelnytskyi et al. (1997) J. Biol. Chem. 272, 28107-28115; Tsukazaki et al. (1998) Cell 95, 779-791; Wrana et al. (1994) Nature 370, 341-347; Zhang et al. (1997) Curr. Biol. 7, 270-276; Zhang et al. (1998) Nature 394, 909-913; Zhou et al. (1998) Mol. Cell 2, 121-127.

Calcium

The bivalent cation Ca^{2+} is, along with cAMP, one of the two major second messengers in eukaryotic cells. Its intracellular concentration is tightly regulated and usually kept very low compared to the cell's environment. Ca^{2+} binding proteins and transporters (Gap junction, Voltage-gated, second messenger-gated) help to sequester huge amounts of the ion in various organelles from where Ca^{2+} can be released upon extracellular stimuli. E.g. the contraction of the muscle is dependent on the presence of Ca^{2+} ions which are readily transported back into the organelles in order for the muscle to relax. In signal transduction, Ca^{2+} functions as a second messenger that activates Ca^{2+} dependent processes through the activation of Ca^{2+}/calmodulin dependent protein kinases (CaM kinases) which are the major effector molecules of Ca^{2+}. In the signaling cascades, the CaM dependent kinases activate phospholipases (e.g. phospholipase C) that in return activate other protein kinases such as protein kinase C.

Rab proteins

In eukaryotic cells the compartmentalization of processes is a prerequisite for a tight regulation of processes and activities. The cells contain a highly dynamic set of membrane compartments that are responsible for packaging, sorting, secreting, and recycling proteins and other molecules. Trafficking between organelles within the secretory pathway occurs as

vesicles derived from a donor compartment fuse with specific acceptor membranes, resulting in the directional transfer of cargo molecules. This process is tightly controlled by the Rab/Ypt family of proteins (reviewed by Novick and Zerial, 1997), a branch of the superfamily of small GTPases. Rab proteins regulate a variety of functions, including vesicle translocation and docking at specific fusion sites. Rabs may also play critical roles in higher order processes such as modulating the levels of neurotransmitter release in neurons, a likely mechanism in synaptic plasticity that underlies learning and memory (Geppert and Südhof, 1998).

Small GTPases share a common three-dimensional fold that, in the GTP bound state, can bind a variety of downstream effector proteins. GTP hydrolysis leads to a conformational change in the "switch" regions that renders the GTPase unrecognizable to its effectors. In this way, by localizing and activating a select set of effectors, a common structural motif is used to control a wide array of distinct cellular processes.

The final steps in membrane fusion are likely to be driven by a set of proteins known as SNAREs. After a vesicle becomes docked, the cytoplasmic domains of VAMP (also termed synaptobrevin) and syntaxin on opposing membranes, in combination with a SNAP-25 molecule, coalesce into an elongated -helical bundle (Poirier et al., 1998 ; Sutton et al., 1998), which may lead to fusion. Because numerous SNARE isoforms have been identified that localize to distinct membrane compartments, it was originally proposed that the specificity of interaction between the SNARE proteins accounted for the specificity in membrane trafficking. Recent results, however, suggest that SNAREs are not specific in their ability to form complexes in vitro, suggesting that trafficking specificity requires additional factors (Yang et al., 1999). In this regard, Rab proteins are strong candidates for governing the specificity of vesicle trafficking. Like the SNAREs, many isoforms (40) of the Rab family have been identified that localize to specific membrane compartments (reviewed by Novick and Zerial, 1997).

Concomitant with the SNARE cycle, Rab proteins undergo a intricate cycle of membrane and protein interactions. Rabs are posttranslationally modified at C-terminal cysteines by the addition of two geranylgeranyl groups, which mediate membrane association when the Rab is in the GTP-bound state. After guanine nucleotide hydrolysis occurs, the Rab is extracted from the membrane upon forming a complex with a cytosolic GDP-dissociation inhibitor (GDI). This cytosolic intermediate is then recycled onto a newly forming vesicle,

most likely through a secondary factor termed a GDI dissociation factor (GDF), which displaces GDI. After the Rab becomes membrane bound, a guanidine nucleotide exchange factor (GEF) promotes release of GDP and the subsequent loading of GTP. In its GTP-bound conformation, the Rab is then free to associate with its specific set of effectors, which can in turn trigger events leading to the eventual fusion of the vesicle with a target membrane. To complete the cycle, perhaps after or concurrent with membrane fusion, a GTPase activating protein (GAP) accelerates nucleotide hydrolysis, switching off the GTPase. The remaining GDP-bound Rab can then participate in a new round of fusion.

Rab interactions with effectors are likely to regulate vesicle targeting and membrane fusion in three ways. First, a Rab may specifically facilitate vectorial vesicle transport. Vesicles are transported from their site of origin to acceptor compartments likely through associations with cytoskeletal elements and transport motors. A protein has been identified with a domain structure that suggests a connection between the cytoskeleton and the Rabs. This protein, called Rabkinesin-6, contains a kinesin-like ATPase motor domain followed by a coiled-coil stalk region and a RBD that specifically binds Rab6 (Echard et al., 1998). An additional link with the cytoskeleton is provided by the Rab effector, Rabphilin-3A. Rabphilin-3A has been shown in vitro to interact with -actinin, an actin-bundling protein, but only when not bound to Rab3A (Kato et al., 1996). These results raise the intriguing possibility that Rab proteins regulate vesicle interactions with the cytoskeleton and thereby play an active role in targeting vesicles to their appropriate destinations.

Second, Rab proteins may regulate membrane trafficking at the vesicle docking step. A number of Rab effectors, including Rabaptin-5, EEA1, Rabphilin-3A, and Rim, may serve as molecular tethers. Each effector protein contains a RBD, followed by a linker region (some having the potential to form elongated coiled-coil structures), and a domain capable of interacting with a second Rab or the target membrane. Rabaptin-5, for example, contains two RBDs, one near the N terminus that specifically recognizes Rab4 and a second near the C terminus that binds Rab5 (Vitale et al., 1998). Both Rim, which is localized to the target membrane, and Rabphilin-3A, which is localized to the vesicle, contain N-terminal RBDs and C-terminal Ca2+-binding C2 domains, implicating these effectors in synaptic vesicle localization or docking in response to Ca2+ influx (Wang et al., 1997). Tethering effectors may also recognize protein complexes on the acceptor membrane. Sec4p, a yeast Rab3A homolog, interacts with the exocyst (Guo et al., 1999), a complex of seven or more subunits

that is assembled at sites of vesicle fusion along the plasma membrane. The exocyst complex may therefore function as a landmark for Rab/effector-mediated vesicle docking.

Third, once a vesicle has become tethered to its fusion site, Rab proteins may selectively activate the SNARE fusion machinery. The mechanism of this activation is unknown but may involve direct interactions of Rabs or, more likely, their effectors with SNAREs. For example, Hrs-2 is a protein that binds to SNAP-25 and contains a Zn2+-finger motif characteristic of Rab-binding proteins such as Rabphilin-3A, Rim, EEA1, and Noc2, suggesting that Hrs-2 may form a physical link between Rabs and SNAREs (Bean et al., 1997). In addition, certain mutations in the syntaxin-binding protein Sly1p, the Sec1p homolog utilized in ER to Golgi trafficking, eliminate the requirement for Ypt1p, a Rab protein that functions at this trafficking step (Dascher et al., 1991). Rabs may therefore regulate SNARE associations through Sec1 family members. In support of this idea, a Rab effector was recently found to interact with a vacuole Rab, a Sec1p homolog, and a SNARE protein (Peterson et al., 1999), which suggests that this effector serves to connect Rab and SNARE function. In this way, Rabs and their effectors may facilitate the correct pairing of SNAREs.

References: Dascher et al. (1991). Mol. Cell. Biol. 11, 872-885; Echard et al. (1998). Science. 279, 580-585; Geppert et al. (1998). Annu. Rev. Neurosci. 21, 75-95; Guoet al. (1999). EMBO J. 18, 1071-1080; Kato et al. (1996). J. Biol. Chem. 271, 31775-31778; Novick et al. (1997). Curr. Opin. Cell Biol. 9, 496-504; Peterson et al. (1999). Curr. Biol. 9, 159-162; Poirier et al. (1998). Nat. Struct. Biol. 5, 765-769; Vitale et al. (1998). EMBO J. 17, 1941-1951; Wang et al. (1997). Nature. 388, 593-598; Yang et al. (1999). J. Biol. Chem. 274, 5649-5653.

Kinases

Reversible posttranslational modifications of proteins are major means of regulating cellular activities. Among the various modifications that are carried out by the cells, the addition of phosphoryl groups to Ser/Thr or Tyr residues is the most important and widely used. The phosphorylation of proteins is accomplished by protein kinases, while the reverse reaction, the removal of phosphoryl groups, is carried out by phosphatases. Kinases / Phosphatases regulate key positions e.g. in the processes of cell proliferation, differentiation and communication/signaling. These processes must be tightly regulated in order to maintain a steady state level of cellular fate. Mis-regulation of kinase activities (or that of

phosphatases) is made responsible for a multitude of disease processes such as oncogenesis, inflammatory processes, arteriosclerosis, and psoriasis.

Protein kinases constitute the largest protein family that is currently known. Several hundred kinases have been identified already. Classically, kinases are subdivided into two classes based on the amino acid residues in their substrates that are phosphorylated by the particular enzymes. The kinases specifically add phosphoryl groups from adenosine triphosphate (ATP) or, less frequently, guanosine triphosphate (GTP), either to serine and/or threonine or to tyrosine residues of substrate proteins. An estimated 1,000 to 10,000 proteins present in a typical mammalian cell are believed to be regulated also by the action of protein kinases.

Protein kinases are frequently integral parts of signaling cascades that transmit extracellular stimuli (e.g. hormones, neurotransmitters, growth- or differentiation factors) into the cell and result in various responses by the cells. The kinases play key roles in these cascades as they constitute a sort of 'molecular switches' turning on or off the activities of other enzymes and proteins, e.g. metabolic, regulatory, channels and pumps, receptors, cytoskeletal, transcription factors.

The regulation of kinase activities is accomplished by various means:

The best characterized example for the regulation via regulatory subunits is the cAMP-dependent protein kinase (PKA) which is also a prototype for second messenger activated protein kinases. This enzyme consists of a heterotetramer of two catalytic (C) and two regulatory (R) subunits. Upon binding of two molecules of second messenger (cAMP) in each R subunit, the catalytic subunits are released and active. Both of the catalytic and the regulatory subunits several isoforms exist. The combination of catalytic and regulatory subunits determines the localization of the holoenzyme and also the substrate spectrum that is available for phosphorylation. The consensus pattern necessary to be present in the substrate for PKA action is RRXS/T where X can be any amino acid.

The casein kinase II comprises another examples for holoenzymes that consist of catalytic and regulatory subunits. Other kinases that are activated by second messengers are cGMP-dependent protein kinase and Protein kinase C (PKC) which is activated by diacylglycerol, which in turn is produced by phospholipases by cleavage of phosphatidylcholine.

Receptor kinases usually consists of an extracellular domain which can bind effector molecules (e.g. growth factors and hormones) and transfer the stimulus to the intracellular domain of these proteins which usually is a protein tyrosine kinase. Other tyrosine kinases lack an extracellular domain but are associated with receptors which transfer the signal after effector binding by activating the associated protein kinase enzyme (e.g. Src kinase family; Src, Blk, Fgr, Fyn, Lck Lyn, Yes and Janus kinase family; Jak1-3, Tyk2).

Dysfunction of kinases, e.g. caused by non-functioning regulation, can be the cause of inflammatory diseases and uncontrolled proliferation. v-Src which is a truncated version of the C-Src protooncogene tyrosine kinase is a classical example for this process as v-Src does not contain the regulatory domain of the cellular gene and is thus constitutively active.

Several categories of proteins are coded for by clones of the invention within the overall group of "Signal transduction"and include, among others, the following:

Neurocalcin (Recoverin): Neurocalcin is a Ca(2+)-binding protein with three putative Ca(2+)-binding domains (EF-hands). In cattle, 6 isoforms are differentially expressed in the central nervous system, retina and adrenal gland. Homology with recoverin indicates involvement in Ca2+ dependent activation of guanylate cyclase.. These proteins can find application in modulating/blocking the guanylate cyclase-pathway. Diseases associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with these proteins include as reported by OMIN 1) autosomal dominant cone dystrophy (OMIN *600364); 2) cone dystrophy 3 (OMIN *600364); 3) cancer associated retinopathy (OMIN *179618). Clones in this category include: fbr2_23b21.

Proteins with a WW Domain: Proteins that contain a WW domain which has been originally described as a short conserved region in a number of unrelated proteins, among them dystrophin, the gene responsible for Duchenne muscular dystrophy. The domain, which spans about 35 residues, is repeated up to 4 times in some proteins. It has been shown to bind proteins with particular proline-motifs, [AP]-P-P-[AP]-Y, and thus resembles somewhat SH3 domains. This domain is frequently associated with other domains typical for proteins in signal transduction processes. Examples of proteins containing the WW domain are Dystrophin, Utrophin, vertebrate YAP protein (binds the SH3 domain of the Yes oncoprotein), murine NEDD-4 (embryonic development and differentiation of the central nervous system), IQGAP (human GTPase activating protein acting on ras). Therefore these proteins should be involved in intracellular signal transduction. Diseases associated (as

potentially diagnostic, therapeutic, causative, and/or related, etc...) with these proteins include as reported by OMIN 1) Muscular Dystrophy, Pseudohypertrophic Progressive Duchenne and Becker Types (OMIN *310200). Clones in this category include: fbr2_23n16.

Protein substrates for cAMP-dependent protein kinase: Acting as a choride channel or chloride channel inhibitor these proteins have been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) as reported by OMIN with Cystic Fibrosis (OMIN #219700). Clones in this category include fbr2_82i17.

Sphingosine kinase: Sphingosine kinase is a new type of lipid kinase, which is regulated by growth factors. The enzyme phosphorylates sphingosine, which subsequently exerts intracellular and extracellular actions. Intracellulary, sphingosine 1-phosphate (SPP) promotes proliferation and inhibits apoptosis. In yeast, survival of cells exposed to heat shock indicates is dependent on SPP. Extracellulary, SPP inhibits cell motility and influences cell morphology, effects that appear to be mediated by the G protein-coupled receptor EDG1. These proteins have been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) as reported by OMIN with Gaucher Disease, Type I (OMIN *230800). Clones in this category include fbr2_82m6.

Vanilloid Receptors: VR1 seems to play an important role in the activation and sensitization of nociceptors. It is the receptor for e.g. capsaicin, a selective activator of nociceptors, a natural product of capsicum peppers. Related can find application as a target for the development of new nociception-modulating drugs. Clones in this category include tes3_20k2.

RCC1 (Regulator of chromosome condensation): RCC1 (regulator of chromosome condensation) is a eukaryotic protein which binds to chromatin and interacts with ran, a nuclear GTP-binding protein. RCC1 promotes the exchange of bound GDP with GTP, acting as a guanine-nucleotide dissociation stimulator. These proteins can find application in the regulation of gene expression by activition of nuclear GTP-binding proteins. The X-linked retinitis pigmentosa is a result of a defect GTPase regulator, which contains a RCC1-type repeat. OMIN also reports that RCC1 has associations (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with retinitis pigmentosa (OMIN *312610). Clones in this category include tes3_21d4.

Ras inhibitor proteins: Ras is a signal transducting molecule involved in the receptor tyrosine kinase/RAS/Map kinase signalling cascade. Ras proteins bind GDP/GTP and show

intrinsic GTPase activity. Mutations in ras, which change aa 12, 13 or 61 activate the potential of ras to transform cultured cells and are implicated in a variety of human tumours. Ras inhibitor proteins have been associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with many disease processes as reported by OMIN including: 1) Tumors of the lung, breast, brain, pituitary, pancrase, bone, skin, bladder, kidney, ovary, prostate and lymphocyte, Melanoma (OMIN *600160); 2) X-linked non-specific mental retardation (OMIN *300104); 3)adenomatouspolyposis of the colon (OMIN *175100); 4) Beckwith-Wieddemann Syndrome (#130650); and 5) Major affective disorder 1 (OMIN *125480). Clones in this category include utel_22g21.

Mammalian proteins cornicon involving the EGF-receptor: Cornicon proteins are part of a signal transduction pathway involving the EGF-receptor. The EGF-receptor has been reported by OMIN to be associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) Familial hypercholesterolemia (OMIN 143890); 2) Leprechaunism (OMIN #246200); 3) Hemophilia B (OMIN *306900); 4) Ectodermal dysplasia 1; 5) Kartagenerer syndrome (OMIN *244400) and 6) Glioma of the brain (OMIN *137800).). Clones in this category include utel_22e12.

Transmembrane proteins

Membrane region prediction was effected using the ALOM2 software (Klein et al., 1985; version 2 by K. Nakai). Similar to many other methods, the Kyte & Doolitle (1982) amino acid hydrophobicity scale is used in ALOM2 as the primary variable for classifying sequences in terms of their localization. High prediction accuracy is achieved through the system of intelligent decision rules and the utilization of a carefully selected training data set. The method also generates reliability estimates which makes it possible to distinguish between membrane-spanning proteins (I, intrinsic) and globular proteins with regions of high hydrophobicity buried in the core.

For a protein of length L, the block of length l with maximum hydrophobicity is found:

$$\max H = \max_{k=1,\ldots,L-l+1}(1/l)\sum_{i=k}^{k+l-1} H_i$$

where H_i represents the hydrophobicity of an individual residue.

Let P(I/maxH) and P(E/maxH) be the conditional probabilities that a protein is integral or peripheral, respectively, given its value of maximal hydrophobicity maxH, and let P(I) and P(E) be the prior probabilities of intrinsic and extrinsic membrane proteins estimated from the training set. Then a sequence is assigned to E if

P(E/maxH) > P(I/maxH)

or, after applying the Bayes rule,

P(E)P(maxH/E) > P(I)P(maxH/I),

where the conditional probabilities P(maxH/E) and P(maxH/I) can be determined based on the estimates of probability distributions of maxH in both groups.

Discriminant analysis allows to simplify this task by calculating the odds P(E/MaxH):P(I/maxH) as e^b, where b is the left-hand side of a linear or quadratic inequality. For example, for the window of length 17, the protein is allocated to the peripheral category E based on the empirically derived quadratic inequality:

$1.05(\text{maxH})^2+12.30\text{maxH}+17.49 > 0$,

whereas the optimal inequality for assigning membrane proteins (category I) is linear:

$-9.02\text{maxH} + 14.27 > 0$

The odds parameter can be made more or less stringent. For example, one can require odds at least 1:10 for a protein to be classified as integral. This leads to higher selectivity but less sensitivity.

The boundaries of membrane-spanning regions in putative membrane proteins are detected by means of an iterative procedure whereby the most hydrophobic region corresponding to the value maxH is considered to be membrane and removed from the sequence. The classification procedure is then repeated again for the remaining sequence, and, if such a protein is again classified as integral, the next most hydrophobic region is considered.

Reference: Klein, P., Kanehisa, M., DeLisi, C. (1985) The detection and classification of membrane-spanning proteins. *Biochem Biophys Acta* **815**: 468-476

Transcription factors

Purified eukaryotic RNA polymerase II is unable to initiate promoter-specific transcription. A family of factors that collectively confer RNAPII promoter specificity is known as the general transcription factors (GTFs). They include the TATA-binding Protein (TBP) TFIIB, TFIIE, TFIIF and TFI IH. These factors are conserved among all eukaryotes.

RNAPII complexes containing the entire set of GTFs or a subset of GTFs together with other proteins have been isolated from mammalian and yeast cells. Although purified RNAPII and GTFs are sufficient for promoter-specific initiation, this system fails to respond to activators. This is mediated by a further complex termed mediator complex which associates with the carboxy-terminal heptapeptide domain (CTD) of the largest subunit of RNAPII.

Purification of human RNAPII complexes resulted in two distinct forms of human RNAPII after analysis of functional properties. One complex contained chromatin remodeling activities but was devoid of GTFs. The other complex did not contain factors that modify chromatin but contained a subset of SRB/mediator subunits and GTFs and other polypeptides that mediate transcriptional activation, a scenario similar to that reported for yeast.

A complex designated NAT (~2O SU) for negative regulator of transcription contains RNAPII, Cdk8, homologs of the yeast mediator complex as well as Rgrl and Srb1O/11 known as negative regulators of transcription.

A complex with striking similar structural and functional properties to NAT has been identified designated SMCC (~l5 SU) (SRB/mediator coactivator complex), that can also mediate transcriptional activation.

The SMCC complex includes all reported NAT subunits including subunits of the TRAP complex. TRAP is a coactivator complex isolated on the basis of its interaction with the thyroid hormone receptor. Another coactivator complex DRIP, isolated on the basis of its

ability to interact with the vitamin D3 receptor, contains novel subunits as well as subunits of NAT/SMCC and TRAP complexes.

The effects of each of these coactivator complexes is dependent on the TFIID complex. It is not known if the T AF subunits of TFIID are required. It is likely that new coactivator complexes will be uncovered containing both novel and previously defined components.

Beside the huge amount of transcription factors which can be part of the RNAIIP holoenzyme or the coactivator complexes there is an even larger quantity of specific transcription factors binding to promoter elements within the DNA sequences of a given gene leading to activation or repression of transcription. A broad range of cellular responses like differentiation, proliferation, cell death and others are elicited through activating or repressing the transcription of target genes.

There are at least five superclasses of transcription factors:

1. Superclass contains members with characteristic basic domains:

Members are:

Leucine zipper factors, where the basic domain is followed by a leucine zipper of repeated leucine residues at every seventh position. The zipper mediates protein dimerization as a prerequisite for DNA-binding.

Helix-loop-helix factors (bHLH) contain a DNA-binding basic region followed by a motif of two potential amphipathic alpha-helices connected by a loop of variable length also mediating dimerization.

Factors with a combination of Helix-loop-helix and leucine zipper.

Further members of this superclass are NF-l, RF-X, and bHSH like proteins.

2. Superclass comprises factors containing zinc-coordinating DNA-binding domains.

Members are:

Proteins with Cys4 zinc finger of nuclear receptor type, where two such motifs differing in size, composition and function are present in each receptor molecule. Each finger comprises 4 cysteine residues coordinating one zinc ion. The second half including the second cysteine pair has alpha-helix conformation and the helix of the first finger binds to the DNA through the major groove. The sequence between the first two cysteines of the second finger mediates dimerization upon DNA-binding. This class includes the steroid hormone receptors and the thyroid hormone receptor-like factors. Other diverse cys4 zinc fingers have a motif of GATA-type.

Proteins with Cys2His2 zinc finger domain(s). Each finger comprises 2 cysteine and 2 histidine residues coordinating one zinc ion, and in some cases one histidine is replaced by another cysteine. The zinc ion is essential for DNA-binding.

Proteins with Cys6 cysteine-zinc cluster(s). Six cysteine residues coordinate two zinc ions, i. e. two of the thiol groups are coordinating two zinc ions each. Present in many fungal regulators.

Zinc fingers of alternating composition.

3. Superclass contains factors of helix-turn-helix type.

Members are:

Proteins with homeo domains. Homeo domains are three consecutive alpha-helix structures. Helix 3 contacts mainly the major groove of the DNA, some contacts at the minor groove are observed as well. Helix 2 and 3 resemble the helix-turn-helix structure of prokaryotic regulators.

Proteins with Paired box domain(s). This is a DNA-binding domain of approximately 130 amino acid residues. Its N-terminal half is basic, its C-terminal half is highly charged in general. It probably comprises 3 alpha-helices.

Proteins with Fork head / winged helix domain(s). This domain was identified by homology between HNF-3A and fkh. The domain comprises approx. 110 AA. Analysis of the crystal structure has revealed a compact structure of three alpha-helices, the third alpha-helix

being exposed towards the major groove of the DNA. The domain also exerts minor groove contacts. Upon binding to DNA, it induces a bend of 13 degree.

Heat shock factors

Proteins with Tryptophan clusters. The tryptophan clusters comprise several tryptophan residues with a spacing of 12-21 amino acid residues; the subclass of myb-type DNA-binding domains typically exhibit a spacing of 19-21 amino acid residues.

Proteins with TEA domain(s). The TEA domain has been identified as a region which is conserved among the transcription factors TEF-l, TECl and abaA. This domain in TEF-l has been shown to interact with DNA, although two additional regions may also contribute to DNA-binding. lt is predicted to fold into three alpha-helices, with a randomly coiled region of 16-18 amino acid residues between helices 1 and 2, and a short stretch between helices 2 and 3 of 3-8 residues.

4. Superclass contains beta-Scaffold Factors with Minor Groove Contacts

Members are:

Proteins with RHR (Rel homology) region.

The structure of the Rel-type DBD exhibits a bipartite subdomain structure, each subdomain comprising a beta-barrel with five loops that form an extensive contact surface to the major groove of the DNA. Particularly, the first loop of the N-terminal subdomain (the highly conserved recognition loop) performs contacts with the recognition element on the DNA, but other loops are involved. The fact that the main DNA-contacts are made through loops has been suggested to provide a high degree of flexibility in binding to a range of different target sequences. Augmenting interactions are achieved by two alpha-helices within the N-terminal Part that form strong minor groove contacts to the A/T-rich center of the B-element. In p65, the sequence between both alpha-helices is much shorter and even helix 2 is truncated. The second, C-terminal domain is necessary mainly for protein dimerization.

p53 proteins

MADS (MCMl-agamous-deficiens-SRF) box proteins. Proteins of this class comprise a region of homology. The DNA-binding domain also comprises the dimerization capability. In the DNA-bound dimer (shown for SRF), two antiparallel amphipathic alpha-helices (alpha-I), form a coiled coil and are oriented approximately parallel on the minor groove. These helices make minor and major groove contacts, the N-terminal extensions form minor groove contacts. The bound DNA is bent and wrapped around the protein. It exhibits a compressed minor groove in the center and widened minor groove in the flanks.

Beta-Barrel alpha-helix transcription factors.

TATA-binding proteins

HMG proteins

Proteins of this class comprise a region of homology with the chromosomal non-histone HMG proteins such as HMG1. This region comprises the DNA-binding domain which in some instances such as HMG1 mediates sequence-unspecific, in other cases such LEF-1 sequence-specific binding to DNA. This domain exhibits a typical L-shaped conformation made up of 3 alpha-helices and an extended N-terminal extension of the first helix. The latter together with helix 1, which contains a kink, form the long arm of the L, whereas helices 1 and 2 form the short arm. Binding to the minor groove induces a sharp bending of the DNA by more than 90 degree, away from the bound protein. The overall topology of the DNA-protein complexes resembles somewhat that of the TBP-TATA box complex.

Heteromeric CCAAT factors

Proteins with Grainyhead domain(s)

Cold-shock domain factors. Cold-shock domain proteins are characterized by a highly conserved region first found in prokaryotic cold-shock proteins. This domain is a single-stranded nucleic acid-binding structure interacting with DNA or RNA. It consists of an antiparallel five-stranded beta-barrel, the strands of which are connected by turns and loops. Within this structure, a three-stranded beta-strand contains a conserved RNA-binding motif, RNPl. Not all CSD proteins are transcription factors. Those which specifically bind to a

certain sequence are termed Y-box proteins. Proteins of this class were previously called protamine-like domain proteins because of having a highly positively charged domain with interspersed proline residues.

Proteins with Runt homology domain

The members of this transcription factor class have been identified on the basis of their homology to a defined region within the Drosophilia protein Runt. The runt domain is part of the DNA-binding domain of these factors. It consists mainly of beta-strands, does not contain alpha-helical regions and seems to be most similar to the palm domain found in DNA polymerase beta (rat).

5. Superclass contains other transcription factors like Copper fist proteins, HMGI(Y), STAT, Pocket domain proteins and Ap2/EREBP-related factors.

The classification of transcription factors originates from TRANSFAC database:

http: //transfac.gbf.de/TRANSFAC/

Reference: Heinemeyer

Several categories of proteins are coded for by clones of the invention within the overall group of "Transcription Factors".and include, among others, the following:

Dcoh: Dcoh is a bifunctional protein, complexed with biopterin. It serves as dimerization cofactor of hepatocyte nuclear factor-1 and catalyzes the dehydration of the biopterin cofactor of phenylalanine hydroxylase. The Dcoh protein has been reported by OMIN to be associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) hyperphenylalanemia (OMIN 126090, #264070). Clones in this category include fkd2_46k12.

Signal transducing proteins: Beta-transducin subunits of G-proteins contain WD-40 repeats. The beta subunits seem to be required for the replacement of GDP by GTP as well as for membrane anchoring and receptor recognition. Due to the zinc finger the novel protein seems to be a new molecule involved in signal transduction and transcription. These proteins have been reported by OMIN to be associated (as potentially diagnostic, therapeutic, causative, and/or related, etc...) with the following diseases: 1) essential hypertension (OMIN *139130). Clones in this category include utel_1i2.

* * *

The invention, therefore, specifically contemplates the following assemblages of materials, which track the above-identified fourteen functional groupings, that are useful in practicing the profiling aspects of the invention. One type of assemblage is nucleic acid-based and can include the following groupings of sequences and their derivatives: all sequences; human fetal brain sequences; brain derived sequences; human fetal kidney library sequences; kidney derived sequences; human mammary carcinoma library sequences; mammary carcinoma derived sequences; human testis library sequences; testes derived sequences; cell cycle genes; cell structure and motility genes; differentiation and development genes; intracellular transport and trafficking genes; metabolism genes; nucleic acid management genes; signal transduction genes; transmembrane protein genes; and transcription factor genes. Other assemblages contain proteins or their corresponding antibodies or antibody fragments, divided along the same groupings.

Database Applications

Because they are human genes and gene products, the inventive molecules are useful as members of a database. Such a database may be used, for example, in drug discovery and rationale drug design or in testing the novelty and non-obviousness of newly sequenced materials. In addition, they are particularly suited in designing variants for the profiling (and other) applications described herein. Hence, the following discussion of electronic embodiments applies equally to such variants, which, naturally, will be generated and stored using a computer using known methodologies.

Accordingly, one aspect of the invention contemplates a database of at least one of the inventive sequences stored on computer readable media. Again, the individual sequences may be grouped with regard to the individual functional and structural groups mentioned above. While the individual sequences of a database may exist in printed form, they are preferably in electronic form, as in an ascii or a text file. They may also exist as word processing files or they may be stored in database applications like DB2, Sybase, Oracle, GCG and GenBank. One skilled in the art will understand the range of applications suitable for using and storing the electronic embodiments of the invention.

"Computer readable media" refers to any medium which can be read and accessed by a computer. These include: magnetic storage media, like floppy discs, hard drives and magnetic tape; optical storage media, like CD-ROM; electrical storage media, like RAM

and ROM; and hybrids of these categories, like magnetic/optical storage media. One skilled in the art will readily understand the scope of computer readable media and how to implement them.

Biological Activities and Assays for Implementing Therapeutic and Diagnostic Applications

This section provides assays for biological activity that are useful in characterizing and quantifying the biological activity of the inventive molecules and their derivatives, which is relevant to the pharmacological effects of the inventive molecules. As used in this section, it will be understood that "protein" may also refer to the inventive antibodies (including fragments).

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M + (preB M +), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin gamma , Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Aced. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11-Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9-Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by vital (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to modify immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the

tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-vital immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient.

The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and beta 2 microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of

Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the

treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendonitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and

cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle

stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin alpha family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- beta group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of

cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such

receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of

cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in

a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

Particular Applications for Certain Clones

The following sets out a non-exclusive list of applications for certain embodiments of the invention. In the interest of economy, applications relevant to multiple embodiments are not duplicated in this list. Other embodiments described in below have similar characteristics, as described therein. The artisan is directed, therefore, to this section for similar descriptions of the functions of other embodiment.

Testes

htes3_15c24: The new protein can find application in modulation of 2-hydroxyacid dehydrogenases-dependent pathways and as a new enzyme for biotechnologic production processes.

htes3_15i5: The new protein can find application in modulating the structure of the human spermatozoa radia spoke head and modulation of sperm motility in men.

htes3_15k11: The novel protein contains a protein kinase ATP-binding region signature and a serine/threonine protein kinase active-site signature. The new protein can find application in modulation of intracellular signal pathways dependent on this kinase.

htes3_17n12: The new protein can find application in modulating/blocking the expression of SOX-controlled genes.

htes3_20k2: The new protein can find application as a target for the development of new nociception-modulating drugs.

htes3_20m18: The new protein can find application in modulation of mitochondrial DNA replication and maintenance.

htes3_20d4: The new protein can find application in the regulation of gene expression by activition of nuclear GTP-binding proteins. The X-linked retinitis pigmentosa is a result of a defect GTPase regulator, which contains a RCC1-type repeat.

htes3_21j15: NY-CO-33 is a protein recognised by autologous antibodies of human colon cancer patients. The novel protein contains 4 C2H2 Zinc fingers and is a new putativ transcription factor. The new protein can find application in modulating/blocking the expression of genes controlled by this transcription factor.

The new protein can find application in modulating chromosome transport in mitosis and meiosis and modulation of cell division.

htes3_26g22: The new protein can find application in modulating chromosome transport in mitosis and meiosis and modulation of cell division. The novel TBP-binding protein is considered to participate in transcription regulation through the interaction with TBP. The new protein can find application in modulation of gene transcription.

htes3_21l16: The new protein can find application in modulation of protein translocation into the endoplasmic reticulum.

htes3_27d1: The novel protein can find application in modulation of ubiquitin- and protein metabolism in cells.

htes3_2m18: The novel protein can find application as multifunctional nuclease / exoribonuclease.

htes3_35b4: The new protein can find application in modulation of the mitotic spindle.

htes3_35b5: The novel protein can find application in modulating the v-ATPase activity in endocytic and secretory organelles.

htes3_35e21: Due to the close relationship to human interleukin-7, the novel interleukin is expected to act as a new growth factor for human B lineage cells. Additionally, the protein should induce the gene rearrangement of the T-cell receptor repertoire, leading to thymocyte commitment, and subsequently induce both cytotoxic T-cell- and lymphocyte-activated killer cells. This new interleukin could find clinical application in a variety of conditions of hematolymphopoietic failure and different tumours, because of its recruitment of B cell lineage cells, cytotoxic T-cell- and lymphocyte-activated killer cells.

htes3_35k16: Therefore it is a new fatty acid-CoA synthetasese/ligase with unknown substrate. The new protein can find application in modulation of fatty acid metabolism and as a new enzyme for biotechnologic production processes.

htes3_35n12: The new protein can find application in modulation of ADP-transport and energy metabolism in cells/mitochondria.

htes3_35n9: The new protein can find application in modulation of carboxylester metabolism and as a new enzyme for biotechnologic production processes.

htes3_35p22: The novel protein is closely raleted to human tre-2 and other enzymes involved in the degradation of ubiquitinated proteins. The human tre-2 oncogene encodes a deubiquitinating enzyme, indicating a role for the ubiquitin system in mammalian growth control. The novel protein can find application in cancer diagnostics and treatment, and in regulating protein stability and growth control via regulation of ubiquitination.

htes3_4h6: The novel kinesin protein can find application in modulating the function of kinesin and modulating intracellular transport via/on microtubules.

htes3_72k15: FGD1-related F-actin-binding protein (Farbin/FGD1) is a novel F-actin-binding protein. The gene locus fgd1 seems to be responsible for faciogenital dysplasia or Aarskog-Scott syndrome. Frabin binds F-actin and shows F-actin-cross-linking activity. Overexpression of frabin in Swiss 3T3 cells and COS7 cells induces cell shape change and c-Jun N-terminal kinase activation, as described for FGD1. Because FGD1 has been shown to serve as a GDP/GTP exchange protein for Cdc42 small G protein, it is likely that frabin is a direct linker between Cdc42 and the actin cytoskeleton. Cdc42p is an esin yeast, Cdc42p transduces signals to the actin cytoskeleton to initiate and maintain polarized growth and to mitogen-activated protein morphogenesis. In mammalian cells, Cdc42p regulates a variety of actin-dependent events and induces the JNK/SAPK protein kinase cascade, which leads to the activation of transcription factors within the nucleus. The novel protein seems to be the human orthologue of rat frabin.

The new protein can find application in modulating of cell structure and motility as well as modulation of the JNK/SAPK pathway.

htes3_72p16: As Mem3, the novel protein is similar to yeast VPS (vacuolar protein sorting) 35. The null allele of VPS35 results in yeast in a differential defect in the sorting of vacuolar carboxypeptidase Y (CPY), proteinase A (PrA), proteinase B (PrB), and alkaline phosphatase (ALP). The new protein can find application in modulation the sorting of proteins into different compartments.

htes3_7b22: The novel protein is related to paramyosin, a major structural component of thick filaments and invertebrate muscle. Paramyosins are promising antigens for immunization against several parasites, such as Schistosoma mansoni. The new protein can find application in modulating cell adhesion/motility and membrane/cyto skeleton structure and dynamic.

htes3_7j3: The new protein is closely related to C-Tak1 and therefore should be involved in cell-cycle regulation, too. The new protein can find application in modulating/blocking the cell cycle.

htes3_7p9: The nuclear domain (ND)10 also described as POD or Kr bodies is involved in the development of acute promyelocytic leukemia and virus-host interactions. The NDP52 protein is part of this complex structure. In vivo, NDP52 is transcribed in all human tissues, but is redistributed upon viral infection and interferon treatment. ND10 plays an important role in the viral life cycle. The novel protein is similar to NDP52. It contains three leucine zippers and a RGD cell attachment site. This protein seems to be a novel part of the ND819) complex. The new protein can find application in modulation of viral infections and tumour events.

htes3_8m10: The poly(A)-binding protein (PABP) binds to the messenger (mRNA) 3'-poly(A) tail found on most eukaryotic mRNAs and together with the poly(A) tail has been implicated in governing the stability and the translation of mRNA. The new protein can find application in modulation of mRNA translation and processing/stability.

Kidney

hfkd2_24b15: The new protein can find application in modulation of hexose metabolism pathways and as a new enzyme for biotechnologic production processes.

hfkd2_24n20: The new protein seems to be part of the signalling pathway between tyrosine kinases and the membrane/cyto skeleton. The new protein can find application in modulating cell adhesion/motility and membrane/cyto skeleton structure and dynamics.

hfkd2_3o17: The new protein can find application in modulation of the respiratory electron transport chain pathways of mitochondria.

hfkd2_46j20: The new protein can find application in modulating the homoprotocatechuate degradative pathway and as a enzyme for biotechnologic production processes.

hfkd2_46k19: The new protein can find application in modulating/blocking the expression of genes controlled by the hepatocyte nuclear factor-1.

hfkd2_46m4: SAR1 proteins are involved in vesicular transport between the endoplasmic reticulum and the Golgi apparatus.

hfkd2_46k14: rab6 is a ubiquitous ras-like GTPase involved in intra-Golgi transport. The new protein can find application in modulating the transport of vesicles inside the Golgi apparatus.

Uterus Associated:

hutel_18i19: The SREBP-2 protein is embedded in the membranes of the nucleus and endoplasmic reticulum. In cholesterol-depleted cells the proteins are cleaved to release soluble NH2-terminal fragments that enter the nucleus and activate genes encoding the low density lipoprotein receptor and enzymes of cholesterol synthesis. The new protein is a putative transcription factor capable of protein-protein interaction via a lim domain and additionally shows similarity to the common sunflower transcription factor SF3.

hutel_18l1: The novel protein is similar to several 40S ribosomal proteins and therefore seems to part of the corresponding ribosome sub-unit.

hutel_19g22: The new protein can find application in modulation of tissue-calcification, especially the uterus.

hutel_19h17: The new protein can find application in modulating the response of cells to oxysterols.

hutel_20b19: The novel protein seems to be a novel enzyme with sarcosine oxidase activity. The new protein can find application in modulation of sarcosine metabolism and as a new enzyme for biotechnologic production processes.

hutel_20g21: The novel protein seems to be a new ras inhibitor protein. The new protein can find application in modulating/blocking ras dependent signal transduction pathways.

hutel_20h13: The novel protein is a new human alpha-adaptin. The new protein can find application in modulating endocytosis and vesicle trafficking in cells.

hutel_20m11: The new protein can find application in modulating/blocking the activity of protein phosphatase-1 and in modulating the cell cycle.

hutel_20m24: This protein is a putative mannosyl transferase that is involved in the assembly of the core oligosaccharide Glc3Man9GlcNAc2. The new protein can find application in modulation of glycosylation of proteins and as a new enzyme for biotechnologic production processes.

hutel_22e12: The new protein can find application in modulating the cornichon modulated signal transduction way and also the EGF receptor signaling processes.

hutel_23e13: The novel protein contains a serine protease of the subtilase family with an aspartic acid-containing active site. The new protein can find application in modulation of proteinase activity in cells and as a new enzyme for proteomics and biotechnologic production processes.

hutel_24j6: The new protein can find application in modulation of cell-cell-adhesion.

hutel_24h3: The new protein can find application as a useful marker for chondro-osteogenic cell differentiation and for the modulation of chondro-osteogenic cell differentiation.

Fetal Brain:

hfbr2_16c16: The new protein can find application in modulating/blocking of cyto skeleton-membrane protein interaction.

hfbr2_23b21: The new protein can find application in modulating/blocking the guanylate cyclase-pathway.

hfbr2_23b10: The new protein can find application in modulation of splicing.

hfbr2_2b5: The novel protein contains the typical (xxG)n repeat of collagen proteins and a Pfam von Willebrand factor type A domain. Therefore, the protein seems to be a new collagen alpha chain. The new protein can find application in modulation of connective tissue, bone and cartilage development and maintainance.

hfbr2_2c17: The new protein can find application in modulating/blocking G-protein-dependent pathways.

hfbr2_2d15: The new protein can find application in modulating early spermatogenesis.

hfbr2_2i17: The new protein can find clinical application in modulating the transport of glycoproteins inside cells, especially of the LDL receptor.

hfbr2_2k14: Tumour-suppressor genes are known to be involved in the control of cell growth and division, interacting with proteins which control the cell cycle. The N33 gene is significantly methylated in tumour cells, a mechanism by which tumor-suppressor genes are inactivated in cancer. In addition, the novel protein contains a RGD cell attachment site. Therefore the novel protein is a new putative tumour-suppressor gene.

hfbr_3c18: RNA helicases comprise a large family of proteins that are involved in basic biological systems such as nuclear and mitochondrial splicing processes, RNA editing, rRNA processing, translation initiation, nuclear mRNA export, and mRNA degradation. RNA helicases are essential factors in cell development and differentiation, and some of them play a role in transcription and replication of viral single-stranded RNA genomes. The members of the largest subgroup, the DEAD and DEAH box proteins, exhibit a strong dependence of the unwinding activity on ATP hydrolysis. The novel protein contains a DEAD-box and is a new member of this subgroup.

hfbr_3g8: The new protein can find application modulating NAT assembly and action and therefore be important in metabolism of drugs and environmental mutagens.

hfbr2_62b11: The rac small GTPase is associated with type-I phosphatidylinositol 4-phosphate 5-kinase and regulating the production of phosphatidylinositol 4,5-bisphosphate. The new protein is expected to activate p21rac-related small GTPases.

hfbr2_62o17: The new protein can find application in modulation of cholesterol binding and transport by LDL-receptors and LDL-binding proteins.

hfbr_6b24: The new protein can find application in modulation of rhamnose metabolism and as a new enzyme for biotechnologic production processes.

hfbr_72b18: The new protein can find application in modulating DNA repair and mutagenesis.

hfbr_78c4: The new protein can find application in modulating/blocking the response of cells to interferons.

hfbr_78k24: These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquinated proteins. The new protein can find application in modulation of protein stability/degradation in cells.

hfbr_82e4: The new protein can find clinical application in modulating/blocking calmodulin-mediated pathways in human neuronal cells.

VARIANTS OF THE INVENTIVE DNA MOLECULES

Variants in General

"Variants," according to the invention, include DNA and/or protein molecules that resemble, structurally and/or functionally, those set forth in herein. Variants may be isolated from natural sources ("homologs"), may be entirely synthetic or may be based in part on both natural and synthetic approaches.

The section set forth below presents various structural and functional characteristics of molecules within the invention. Preferred molecules are characterized by a combination of one or more of these characteristics. For instance, some preferred molecules are described with reference to at least two structural characteristics, while others may be described with reference to at least one structural and at least one functional characteristic.

It will be recognized by the skilled artisan that structure ultimately defines function, *i.e.* the functions of the molecules described herein derives from the structures of those

molecules. Accordingly, the structural variants described below that bear the closest structural relationship (as variously defined below) to the inventive molecules are the variants that most likely will preserve biological function. This relationship between structure and function will guide the skilled artisan in identifying the preferred embodiments of the invention.

Splicing Variants

It is well-known that eukaryotic structural genes are comprised of both protein coding and non-coding portions. When the messenger RNA is transcribed from the DNA template, it contains introns, which are non-coding, and exons, which are coding. In order to form a translation competent mRNA, the introns must be "spliced" out of this initial pre mRNA.

Specific sequences within the pre mRNA represent "splice junctions" that direct the cellular splicing machinery to the appropriate position. The splice junctions are loosely conserved sequence regions of the pre mRNA, which almost invariably begin with GT and end with AG (DNA perspective). The 5' end of the splice junction typically contains about nine somewhat conserved residues, for example, C/AAGTA/GAGT. The 3' end usually contains a pyrimidine rich stretch of at least about 11 nucleotides, followed by NC/TAGG. Splicing occurs before the GT and after the AG. Mount, *Nucleic Acids Res.* 10:459-72 (1982).

Interestingly, exons often correspond to discrete functional domains of the protein product. The intron/exon arrangement thus creates a linear array of nucleotides which can be correlated to discrete, and often interchangeable, functional protein fragments. Go, *Nature* 291:90-92 (1981); Branden *et al.*, *EMBO J.* 3:1307-10 (1984). This linear arrangement creates the possibility of generating multiple different full length proteins by rearranging the order of the different functional portions in the array. For example, if a set of exons are arranged 1-2-3-4, where (-) represents the introns separating the exons, a splicing event need not simply produce 1234, but may produce 123, 134, 124 and so on. Production of different mRNA products in this way is commonly called "alternative splicing." Andreadis *et al.*, *Ann. Rev. Cell Biol.* 3:207-42 (1987).

Some of the present DNA molecules can be represented in modular fashion in terms of their coding regions. Essentially, these modules are exons (though each "exon" may in fact be made up of several exons), which may be combined in different ways to form a variety of

different DNA molecules, each encoding a different functional protein. Splicing variants are indicated below.

Degenerate Variants

One aspect of the present invention provides "degenerate variants" of the nucleic acid fragments of the present invention. A "degenerate variant" is a nucleotide fragment which differs from those of inventive molecules by nucleotide sequence, but due to the degeneracy of the genetic code, encodes an identical polypeptide sequence.

Given the known relationship between DNA sequences and the proteins they encode, degenerate variants typically are described by reference to this relationship. It is well known that the degeneracy of the genetic code results in many possible DNA sequences which encode a particular protein. Indeed, of the three bases which comprise an amino acid-encoding triplet, the third position, and often the second, almost always may vary. This fact alone allows for a class of variant DNA molecules which encode protein sequences identical to those disclosed herein, yet have about 30% sequence variation. In other words, the variant DNA molecules are about 70% identical to the inventive DNAs, having no additional or deleted sequences. Thus, one aspect of the invention provides degenerate variant DNA molecules encoding the inventive protein sequences.

In one embodiment, these variants have at least about 70% sequence identity with the DNA molecules described herein. In a preferred embodiment, these variants have at least about 80% sequence identity to the inventive molecules. In a more preferred embodiment these variants have at least about 90% sequence identity with the inventive molecules.

Conservative Amino Acid Variants

Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, *i.e.* "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example: (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine;

(c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α-helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β-pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants.

As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences. "Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions.

Functionally Equivalent Variants

Yet another class of DNA variants within the scope of the invention may be described with reference to the product they encode. As shown below, some of the inventive DNA molecules encode a protein having a degree of homology with known proteins, or protein domains. It is expected, therefore, that they will have some or all of the requisite functional features of such molecules. These "functionally equivalent variants" products are characterized by the fact that they are functionally equivalent, with respect to biological activity, to certain known molecules.

The instant invention provides information on common structural motifs, including consensus sequences that will guide the artisan in constructing functionally equivalent variants. It will be understood that the motifs, identified for each inventive protein, may be modified within the identified consensus sequences. Thus, the invention contemplates the proteins disclosed herein that contain variability in the consensus sequences identified, and the invention further contemplates the full range of nucleic acids encoding them, and the complements of those nucleic acids.

Hybridizing Variants

DNA variants within the invention also may be described by reference to their physical properties in hybridization. One skilled in the field will recognize that DNA can be used to identify its complement and, since DNA is double stranded, its equivalent or homolog, using nucleic acid hybridization techniques. It will also be recognized that hybridization can occur with less than 100% complementarity. However, given appropriate choice of conditions, hybridization techniques can be used to differentiate among DNA sequences based on their structural relatedness to a particular probe. For guidance regarding such conditions see, for example, Sambrook *et al.*, 1989, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, N.Y.; and Ausubel *et al.*, 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Green Publishing Associates and Wiley Interscience, N.Y.

Structural relatedness between two polynucleotide sequences can be expressed as a function of "stringency" of the conditions under which the two sequences will hybridize with one another. As used herein, the term "stringency" refers to the extent that the conditions disfavor hybridization. Stringent conditions strongly disfavor hybridization, and only the most structurally related molecules will hybridize to one another under such conditions. Conversely, non-stringent conditions favor hybridization of molecules displaying a lesser degree of structural relatedness. Hybridization stringency, therefore, directly correlates with the structural relationships of two nucleic acid sequences. The following relationships are useful in correlating hybridization and relatedness (where T_m is the melting temperature of a nucleic acid duplex):

a. $T_m = 69.3 + 0.41(G+C)\%$

b. The T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatched base pairs.

c. $(T_m)_{\mu 2} - (T_m)_{\mu 1} = 18.5 \log_{10} \mu 2/\mu 1$
where $\mu 1$ and $\mu 2$ are the ionic strengths of two solutions.

Hybridization stringency is a function of many factors, including overall DNA concentration, ionic strength, temperature, probe size and the presence of agents which disrupt hydrogen bonding. Factors promoting hybridization include high DNA

concentrations, high ionic strengths, low temperatures, longer probe size and the absence of agents that disrupt hydrogen bonding.

Hybridization usually is done in two stages. First, in the "binding" stage, the probe is bound to the target under conditions favoring hybridization. Stringency is usually controlled at this stage by altering the temperature. For high stringency, the temperature is usually between 65°C and 70°C, unless short (<20 nt) oligonucleotide probes are used. A representative hybridization solution comprises 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100μg of non-specific carrier DNA. See Ausubel *et al.*, *supra*, section 2.9, supplement 27 (1994). Of course many different, yet functionally equivalent, buffer conditions are known. Where the degree of relatedness is lower, a lower temperature may be chosen. Low stringency binding temperatures are between about 25°C and 40°C. Medium stringency is between at least about 40°C to less than about 65°C. High stringency is at least about 65°C.

Second, the excess probe is removed by washing. It is at this stage that more stringent conditions usually are applied. Hence, it is this "washing" stage that is most important in determining relatedness via hybridization. Washing solutions typically contain lower salt concentrations. One exemplary medium stringency solution contains 2X SSC and 0.1% SDS. A high stringency wash solution contains the equivalent (in ionic strength) of less than about 0.2X SSC, with a preferred stringent solution containing about 0.1X SSC. The temperatures associated with various stringencies are the same as discussed above for "binding." The washing solution also typically is replaced a number of times during washing. For example, typical high stringency washing conditions comprise washing twice for 30 minutes at 55° C. and three times for 15 minutes at 60° C.

The present invention includes nucleic acid molecules that hybridize to the inventive molecules under high stringency binding and washing conditions. More preferred molecules (from an mRNA perspective) are those that are at least 50 % of the length of any one of those depicted in below. Particularly preferred molecules are at least 75 % of the length of those molecules.

Substitutions, Insertions, Additions and Deletions

In a general sense, the preferred DNA variants of the invention are those that retain the closest relationship, as described by "sequence identity" to the inventive DNA molecules. According to another aspect of the invention, therefore, substitutions, insertions, additions and deletions of defined properties are contemplated. It will be recognized that sequence

identity between two polynucleotide sequences, as defined herein, generally is determined with reference to the protein coding region of the sequences. Thus, this definition does not at all limit the amount of DNA, such as vector DNA, that may be attached to the molecules described herein. Preferred DNA sequence variants include molecules encoding proteins sharing some or all of any relevant biological activity of the native molecule.

In creating these variants, the skilled worker will be guided by reference to the protein structure. First, insertions and deletions in any recognized functional domain, above, generally should be avoided, except as noted below in the section entitled "Proteins," where this domain is discussed in detail. Alterations in such domains usually will be limited to conservative amino acid substitutions. In addition, where insertions and deletions are desired, this may be accomplished at the N- and/or C-terminus of the protein molecule (or the corresponding coding regions of the DNA). If insertions or deletions are made within the protein, deletions of major structural features usually should be avoided. Thus, a preferred place to make insertion or deletion variants is in non-structural regions, such as linker regions between two alpha helices.

"Substitutions" generally refer to alterations in the DNA sequence which do not change its overall length, but only alter one or more nucleotide positions, substituting one for another in the common sense of the word. One class of preferred substitutions, "degenerate substitutions," are those that do not alter the encoded amino acid sequence. Some subsitutions retains 50%, 55%, 60% or 65% identity. Preferred substitutions retain at least about 70% identity, more preferably at least 70% or 75% identity, with the inventive DNAs. Some more preferred molecules have at least about 80% identity, more preferably at least 80% or 85% identity. Particularly preferred DNAs share at least about 90% identity, more preferably at least 90% or 95% identity.

"Insertions," unlike substitutions, alter the overall length of the DNA molecule, and thus sometimes the encoded protein. Insertions add extra nucleotides to the interior (not the 5' or 3' ends) of the subject DNAs. Preferred insertions are made with reference to the protein sequence encoded by the DNA. Thus, it is most preferred to provide an insertion in the DNA at a location that corresponds to an area of the encoded protein which lacks structure. For instance, it typically would not be beneficial, if the preservation of biological activity is desired, to provide an insertion within an alpha-helical region or a beta-pleated sheet. Accordingly, non-structural areas, such as those containing helix-breaking glycines

and proline residues, are most preferred sites of insertion. Other preferred sites of insertion are the splice sites, which are indicated above in the description of the inventive DNA molecules.

While the optimal size of insertions will vary depending upon the site of insertion and its effect on the overall conformation of the encoded protein, some general guides are useful. Generally, the total insertions (irrespective of their number) should not add more than about 30% (or preferably not more than 30%) to the overall size of the encoded protein. More preferably, the insertion adds less than about 10-20% (yet more preferably 10-20%) in size, with less than about 10% being most preferred. The number of insertions is limited only by the number of suitable insertions sites, and secondarily by the foregoing size preferences.

"Additions," like insertions, also add to the overall size of the DNA molecule, and usually the encoded protein. However, instead of being made within the molecule, they are made on the 5' or 3' end, usually corresponding to the N- or C- terminus of the encoded protein. Unlike deletions, additions are not very size-dependent. Indeed, additions may be of virtually any size. Preferred additions, however, do not exceed about 100% of the size of the native molecule. More preferably, they add less than about 60 to 30% to the overall size, with less than about 30% being most preferred.

"Deletions" diminish the overall size of the DNA and, therefore, also reduce the size of the protein encoded by that DNA. Deletions may be made from either end of the molecule or internal to it. Typical preferred deletions remove discrete structural features of the encoded protein. For example, some deletions will comprise the deletion of one or more exons which may define a structural feature. Preferred deletions remove less than about 30% of the size of the subject molecule. More preferred deletions remove less than about 20% and most preferred deletions remove less than about 10%.

Computer-Defined Variants and Definition of "Sequence Identity"

In general, both the DNA and protein molecules of the invention can be defined with reference to "sequence identity." As used herein, "sequence identity" refers to a comparison made between two molecules using, for example, the standard Smith-Waterman algorithm that is well known in the art.

Some molecules have at lease about 50%, 55% or 60% identity. Preferred molecules are those having at least about 65% sequence identity, more preferably at least 65% or 70% sequence identity. Other preferred molecules have at least about 80%, more preferably at

least 80% or 85%, sequence identity. Particularly preferred molecules have at least about 90% sequence identity, more preferably at least 90% sequence identity. Most preferred molecules have at least about 95%, more preferably at least 95%, sequence identity. As used herein, two nucleic acid molecules or proteins are said to "share significant sequence identity" if the two contain regions which possess greater than 85% sequence (amino acid or nucleic acid) identity.

"Sequence identity" is defined herein with reference the Blast 2 algorithm, which is available at the NCBI (http://www.ncbi.nlm.nih.gov/BLAST), using default parameters. References pertaining to this algorithm include: those found at http://www.ncbi.nlm.nih.gov/BLAST/blast_references.html; Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. (1993) "Identification of protein coding regions by database similarity search." Nature Genet. 3:266-272; Madden, T.L., Tatusov, R.L. & Zhang, J. (1996) "Applications of network BLAST server" Meth. Enzymol. 266:131-141; Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402; and Zhang, J. & Madden, T.L. (1997) "PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation." Genome Res. 7:649-656.

METHODS OF MAKING VARIANTS

It will be recognized that variants of the inventive molecules can be constructed in several different ways. For example, they may be constructed as completely synthetic DNAs. Methods of efficiently synthesizing oligonucleotides in the range of 20 to about 150 nucleotides are widely available. See Ausubel *et al.*, *supra*, section 2.11, Supplement 21 (1993). Overlapping oligonucleotides may be synthesized and assembled in a fashion first reported by Khorana *et al.*, J. Mol. Biol. 72:209-217 (1971); see also Ausubel *et al*, Section 8.2. The synthetic DNAs are designed with convenient restriction sites engineered at the 5' and 3' ends of the gene to facilitate cloning into an appropriate vector.

An alternative method of generating variants is to start with one of the inventive DNAs and then to conduct site-directed mutagenesis. See Ausubel *et al.*, *supra*, chapter 8, Supplement 37 (1997). In a typical method, a target DNA is cloned into a single-stranded

DNA bacteriophage vehicle. Single-stranded DNA is isolated and hybridized with a oligonucleotide containing the desired nucleotide alteration(s). The complementary strand is synthesized and the double stranded phage is introduced into a host. Some of the resulting progeny will contain the desired mutant, which can be confirmed using DNA sequencing. In addition, various methods are available that increase the probability that the progeny phage will be the desired mutant. These methods are well known to those in the field and kits are commercially available for generating such mutants.

ISOLATING HOMOLOGS

Methods

By using the sequences disclosed herein as probes or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs. "Homologs" are essentially naturally-occurring variants and include allelic, species-specific and tissue-specific variants.

Region-specific primers or probes derived from the nucleotide sequence(s) provided can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding a homolog using known methods (Innis *et al., PCR Protocols,* Academic Press, San Diego, CA (1990)). Such an application is useful in diagnostic methods, as described in more detail below, as well as in preparing full-length DNAs from various sources. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. As a general guide, the formula 3(G+C) + 2(A+T) = °C, is useful.

When using primers derived from the inventive sequences, one skilled in the art will recognize that by employing high stringency conditions (*e.g.*, annealing at 50-60°C), only sequences with greater than 75% sequence identity to the primer will be amplified. By employing lower stringency conditions (e.g., annealing at 35-37°C), sequences which have greater than 40-50% sequence identity to the primer also will be amplified.

The PCR product may be subcloned and sequenced to confirm that it indeed displays the expected sequence identity. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled

and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see e.g., Sambrook et al., 1989, *supra*.

When using DNA probes derived from the inventive sequences for colony/plaque hybridization, one skilled in the art will recognize that by employing medium to high stringency conditions (e.g., hybridizing at 50-65°C in 5X SSPC and 50% formamide, and washing at 50-65°C in 0.5X SSPC), sequences having regions with greater than 90% sequence identity to the probe can be obtained, and that by employing lower stringency conditions (e.g., hybridizing at 35-37°C in 5X SSPC and 40-45% formamide, and washing at 42°C in SSPC), sequences having regions with greater than 35-45% sequence identity to the probe will be obtained.

Suitably, genomic or cDNA libraries can be constructed and screened in accord with the previous paragraph. The libraries should be derived from a tissue or organism that is known to express the gene of interest, or that is suspected of expressing the gene. The clone containing the homolog may then be purified through methods routinely practiced in the art, and subjected to sequence analysis.

Additionally, an expression library can be constructed utilizing DNA isolated from or cDNA synthesized from a tissue or organism that is known to express the gene of interest, or that is suspected of expressing the gene. In this manner, clones may be induced and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal gene product, as described herein. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor Press.)

Human Homologs

Any organism or tissue can be used as the source for homologs of the present invention so long as the organism or tissue naturally expresses such a protein or contains genes encoding the same. The most preferred organism for isolating homologs is human.

PROTEINS OF THE INVENTION

One class of proteins included within the invention is encoded by the inventive DNA molecules presented. Other proteins according to the invention are those encoded by the DNA variants described above. As noted, these variants are designed with the encoded proteins in mind.

A preferred class of protein fragments includes those fragments which retain any biological activity. These molecules share functional features common the family of proteins, although these characteristics may vary in degree.

According to one aspect of the invention fragments of the inventive proteins are contemplated. Some preferred fragments are those which are capable of eliciting an immune response. Generally these "antigenic" fragments will be from about five amino acids in length to about fifty amino acids in length. Some preferred antigenic fragments are from five to about twenty amino acids long. "Antigenic" response may refer to a T cell response, a B cell response or a response by cells of the macrophage/monocyte lineages. In most cases, however, it will refer to the immune response involved in the generation of antibodies. In other words, the relevant immune response is that of helper T cells and/or B cells. These preferred molecules comprise one or more T cell and /or B cell epitopes.

ANTIBODIES OF THE INVENTION

Antibodies raised against the proteins and protein fragments of the invention also are contemplated by the invention. Described below are antibody products and methods for producing antibodies capable of specifically recognizing one or more epitopes of the presently described proteins and their derivatives.

Antibodies include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies including single chain Fv (scFv) fragments, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, epitope-binding fragments, and humanized forms of any of the above.

As known to one in the art, these antibodies may be used, for example, in the detection of a target protein in a biological sample. They also may be utilized as part of treatment methods, and/or may be used as part of diagnostic techniques whereby patients may be tested for abnormal levels or for the presence of abnormal forms of the such proteins.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., *J. Immunol. Methods* 35:1-21 (1980); Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* 4:72 (1983); Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), pp. 77-96). Antibodies may also be generated by the known techniques of phage display and *in vitro* immunization.

Polyclonal Antibodies

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as an inventive protein or an antigenic derivative thereof.

Polyclonal antiserum, containing antibodies to heterogeneous epitopes of a single protein, can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified, as known in the art, to enhance immunogenicity. Immunization methods include subcutaneous or intraperitoneal injection of the polypeptide.

Effective polyclonal antibody production is affected by many factors related both to the antigen and to the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and/or adjuvant. In addition, host animal response may vary with site of inoculation. Both inadequate or excessive doses of antigen may result in low titer antisera. In general, however, small doses (high ng to low μg levels) of antigen administered at multiple intradermal sites appears to be most reliable. Host animals may include but are not limited to rabbits, mice, chickens and rats, to name but a few. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

The protein immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to coupling the antigen with a heterologous protein (such as globulin β-galactosidase) or through the inclusion of an adjuvant during immunization. Adjuvants include Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum.*

Booster injections can be given at regular intervals, with at least one usually being required for optimal antibody production. The antiserum may be harvested when the antibody titer begins to fall. Titer may be determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen. See, for example, Ouchterlony *et al.*, Chap. 19 in: *Handbook of Experimental Immunology,* Wier, ed, Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 μM). The antiserum may be purified by affinity chromatography using the immobilized immunogen carried on a solid support. Such methods of affinity chromatography are well known in the art.

Affinity of the antisera for the antigen may be determined by preparing competitive binding curves, as described, for example, by Fisher, Chap. 42 in: *Manual of Clinical Immunology,* second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D.C. (1980).

In addition to using protein an the immunogen, DNA molecules may be used directly. In this manner, a DNA encoding the protein immunogen is administered. Boosting and harvesting is done in a manner analogous to that detailed above. Yet another method of producing antibodies entails immunizing chickens and harvesting the antibodies from their eggs.

Monoclonal Antibodies

Monoclonal antibodies (MAbs), are homogeneous populations of antibodies to a particular antigen. They may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture or *in vivo*. MAbs may be produced

by making hybridomas which are immortalized cells capable of secreting a specific monoclonal antibody.

Monoclonal antibodies to any of the proteins, peptides and epitopes thereof described herein can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., *Nature* 256:495-497 (1975) (and U.S. Patent No. 4,376,110) or modifications of the methods thereof, such as the human B-cell hybridoma technique (Kosbor *et al.*, 1983, *Immunology Today* 4:72; Cole *et al.*, 1983, *Proc. Natl. Acad. Sci.* USA 80: 2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

In one method a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen are isolated.

The spleen cells are fused, typically using polyethylene glycol, with mouse myeloma cells, such as SP2/0-Ag14 myeloma cells. The excess, unfused cells are destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted, and aliquots are plated to microliter plates where growth is continued.

Antibody-producing clones (hybridomas) are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures. These include ELISA, as originally described by Engvall, *Meth. Enzymol.* 70:419 (1980), western blot analysis, radioimmunoassay (Lutz *et al.*, *Exp. Cell Res.* 175:109-124 (1988)) and modified methods thereof.

Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* BASIC METHODS IN MOLECULAR BIOLOGY, Elsevier, New York. Section 21-2 (1989). The hybridoma clones may be cultivated *in vitro* or *in vivo*, for instance as ascites. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production. Alternatively, hybridoma culture in hollow fiber bioreactors provides a continuous high yield source of monoclonal antibodies.

The antibody class and subclass may be determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)).

MAbs may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. Methods of purifying monoclonal antibodies are well known in the art.

Antibody Derivatives and Fragments

Fragments or derivatives of antibodies include any portion of the antibody which is capable of binding the target antigen, or a specific portion thereof. Antibody derivatives include poly-specific (*e.g.*, bi-specific) antibodies, which contain binding sites specific for two or more different epitopes. These epitopes may be from the same or different inventive molecules or one or more epitope may be from a molecule not specifically disclosed here.

Antibody fragments specifically include $F(ab')_2$, Fab, Fab' and Fv fragments. These can be generated from any class of antibody, but typically are made from IgG or IgM. They may be made by conventional recombinant DNA techniques or, using the classical method, by proteolytic digestion with papain or pepsin. See CURRENT PROTOCOLS IN IMMUNOLOGY, chapter 2, Coligan *et al.*, eds., (John Wiley & Sons 1991-92).

$F(ab')_2$ fragments are typically about 110 kDa (IgG) or about 150 kDa (IgM) and contain two antigen-binding regions, joined at the hinge by disulfide bond(s). Virtually all, if not all, of the Fc is absent in these fragments. Fab' fragments are typically about 55 kDa (IgG) or about 75 kDa (IgM) and can be formed, for example, by reducing the disulfide bond(s) of an $F(ab')_2$ fragment. The resulting free sulfhydryl group(s) may be used to conveniently conjugate Fab' fragments to other molecules, such as detection reagents (*e.g.*, enzymes).

Fab fragments are monovalent and usually are about 50 kDa (from any source). Fab fragments include the light (L) and heavy (H) chain, variable (V_L and V_H, respectively) and constant (C_L C_H, respectively) regions of the antigen-binding portion of the antibody. The H and L portions are linked by an intramolecular disulfide bridge.

Fv fragments are typically about 25 kDa (regardless of source) and contain the variable regions of both the light and heavy chains (V_L and V_H, respectively). Usually, the V_L and V_H chains are held together only by non-covalent interacts and, thus, they readily dissociate. They do, however, have the advantage of small size and they retain the same binding properties of the larger Fab fragments. Accordingly, methods have been developed to crosslink the V_L and V_H chains, using, for example, glutaraldehyde (or other chemical crosslinkers), intermolecular disulfide bonds (by incorporation of cysteines) and peptide linkers. The resulting Fv is now a single chain (*i.e.*, SCFv).

Other antibody derivatives include single chain antibodies (U.S. Patent 4,946,778; Bird, Science 242:423-426 (1988); Huston *et al.*, Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward *et al.*, Nature 334:544-546 (1989)). Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain FV (SCFv).

One preferred method involves the generation of scFvs by recombinant methods, which allows the generation of Fvs with new specificities by mixing and matching variable chains from different antibody sources. In a typical method, a recombinant vector would be provided which comprises the appropriate regulatory elements driving expression of a cassette region. The cassette region would contain a DNA encoding a peptide linker, with convenient sites at both the 5' and 3' ends of the linker for generating fusion proteins. The DNA encoding a variable region(s) of interest may be cloned in the vector to form fusion proteins with the linker, thus generating an scFv.

In an exemplary alternative approach, DNAs encoding two Fvs may be ligated to the DNA encoding the linker, and the resulting tripartite fusion may be ligated directly into a conventional expression vector. The scFv DNAs generated any of these methods may be expressed in prokaryotic or eukaryotic cells, depending on the vector chosen.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab)_2$ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Derivatives also include "chimeric antibodies" (Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1984); Neuberger *et al.*, *Nature*, 312:604-608 (1984); Takeda *et al.*, *Nature*, 314:452-454 (1985)). These chimeras are made by splicing the DNA encoding a mouse antibody molecule of appropriate specificity with, for instance, DNA encoding a human antibody molecule of appropriate specificity. Thus, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. These are also known sometimes as "humanized" antibodies and they offer the added

advantage of at least partial shielding from the human immune system. They are, therefore, particularly useful in therapeutic *in vivo* applications.

Labeled Antibodies

The present invention further provides the above-described antibodies in detectably labeled form. Antibodies can be detectably labelled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling are well-known in the art, for example see (Sternberger *et al.*, *J. Histochem. Cytochem.* 18:315 (1970); Bayer *et al.*, *Meth. Enzym.* 62:308 (1979); Engval *et al.*, *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)). The labeled antibodies of the present invention can be used for *in vitro, in vivo*, and *in situ* diagnostic assays.

Immobilized Antibodies

The foregoing antibodies also may be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro, in vivo*, and *in situ* assays as well as for immunoaffinity purification of the proteins of the present invention.

THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS

The proteins, antibodies and polynucleotides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration,

such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, the compounds and their physiologically acceptable salts and solvate may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they maybe presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.* gelatin for

use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

RECOMBINANT CONSTRUCTS AND EXPRESSION

The present invention further provides recombinant DNA constructs comprising one or more of the nucleotide sequences of the present invention. The recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a DNA or DNA fragment, typically bearing an open reading frame, is inserted, in either orientation.

The gene products encoded by the subject DNAs may be produced by recombinant DNA technology using techniques well known in the art. See, for example, the techniques described in Sambrook et al., 1989, *supra*, and Ausubel et al., 1989, *supra*. Alternatively, the DNA sequences may be chemically synthesized using, for example, synthesizers. See, for

example, the techniques described in OLIGONUCLEOTIDE SYNTHESIS, 1984, Gait, ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety. They may be assembled from fragments and short oligonucleotide linkers, or from a series of oligonucleotides. The are preferably made by RT-PCR methods. The resulting synthetic gene is capable of being expressed in a recombinant vector.

In some cases the recombinant constructs will be expression vectors, which are capable of expressing the RNA and/or protein products of the encoded DNA(s). Thus, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the open reading frame (ORF). The vector may further comprise a selectable marker sequence.

Specific initiation signals may also be required for efficient translation of inserted target gene coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where a target DNA includes its own initiation codon and adjacent sequences is inserted into the appropriate expression vector, no additional translation control signals may be needed. However, in cases where only a portion of an ORF is used, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire target. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner *et al.*, *Methods in Enzymol.* 153:516-544 (1987)). Some appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, *et al., in Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield *et al.*, U.S. Patent No. 5,082,767.

The present invention further provides host cells containing at least one of the DNAs of the present invention. The host cell can be virtually any cell for which expression vectors are available. It may be, for example, a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic

cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis *et al., Basic Methods in Molecular Biology* (1986)).

A wide variety of expression systems are available, such as: yeast (*e.g. Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing the target DNA; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the target DNA sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.* Ti plasmid) containing target DNA coding sequences; or mammalian cell systems (*e.g.* COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

Depending on the system chosen, the resulting product may differ. For example, proteins expressed in most bacterial cultures, *e.g.*, *E. coli,* will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern different from that expressed in mammalian cells.

Vectors

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting selection of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequence, and in one aspect of the invention, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal or C-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

Bacterial Expression

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may, also be employed as a matter of choice.

Bacterial vectors may be, for example, bacteriophage-, plasmid- or cosmid-based. These vectors can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids typically containing elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, GEM 1 (Promega Biotec, Madison, WI, USA), pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pKK232-8, pDR540, and pRIT5 (Pharmacia).

These "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Bacterial promoters include lac, T3, T7, lambda P_R or P_L, trp, and ara.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed/induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the coding sequence may be ligated into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye *et al.* 1985, *Nucleic Acids*

Res. 13:3101-3109; Van Heeke *et al.*, 1989, *J. Biol. Chem.* 264:5503-5509); pET vectors, Studier *et al.*, *Methods in Enzymology* 185: 60-89 (Academic Press 1990); and the like.

Moreover, pGEX vectors may be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and easily can be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety.

In a one embodiment, full length cDNA sequences are appended with in-frame *BamHI* sites at the amino terminus and *EcoRI* sites at the carboxyl terminus using standard PCR methodologies (Innis et al., 1990, *supra*) and ligated into the pGEX-2TK vector (Pharmacia, Uppsala, Sweden). The resulting cDNA construct contains a kinase recognition site at the amino terminus for radioactive labeling and glutathione S-transferase sequences at the carboxyl terminus for affinity purification (Nilsson, *et al.* 1985, *EMBO J.* 4: 1075; Zabeau and Stanley, 1982, *EMBO J.* 1:1217.

Eukaryotic Expression

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell 23*:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Mammalian promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Exemplary mammalian vectors include pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). Selectable markers include CAT (chloramphenicol transferase).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest

may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a target protein in infected hosts. (*E.g.*, See Logan *et al.*, 1984, *Proc. Natl. Acad. Sci.* USA 81:3655-3659).

In one embodiment, cDNA sequences encoding the full-length open reading frames are ligated into pCMVß replacing the ß-galactosidase gene such that cDNA expression is driven by the CMV promoter (Alam, 1990, *Anal. Biochem.* 188: 245-254; MacGregor *et al.*, 1989, *Nucl. Acids Res.* 17: 2365; Norton *et al.* 1985, *Mol. Cell. Biol.* 5: 281).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins.

Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, etc.

For long-term, high-yield production of recombinant proteins in eukaryotic cells, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker.

Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the target protein. Such engineered cell lines may be

particularly useful in screening and evaluation of compounds that affect the endogenous activity of the protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell* 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase(Szybalska *et al.*, *Proc. Natl. Acad. Sci. USA* 48:2026 (1962)), and adenine phosphoribosyltransferase(Lowy, *et al.*, *Cell* 22:817 (1980)) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, *Proc. Natl. Acad, Sci. USA* 77:3567 (1980)); O'Hare, *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan *et al.*, *Proc. Natl. Acad. Sci. USA* 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, *J. Mol. Biol.* 150:1); and hydro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, *Gene* 30:147) genes.

An alternative fusion protein system allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 8972-8976 (1991)). In this system, the gene of interest is subcloned into a vaccinia-based plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The target coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of a target gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (*E.g.*, see Smith et al., 1983, *J. Virol.* 46: 584; Smith, U.S. Patent No. 4,215,051).

While the present proteins can be expressed in recombinant systems, as described above, cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

Purification of Recombinant Proteins

Recombinant proteins produced may be isolated by host cell lysis. This may be followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, like lysozyme and chelators.

If inclusion bodies are formed in bacterial systems, they may be extracted from cell pellets using, for example, detergents, reducing agents, salts, urea, guanidinium chloride and extremes of pH (*e.g.* <4 or >10). If denaturation occurs, protein refolding steps (*e.g.*, dialysis) can be used, as necessary, in completing configuration of the mature protein. If disulfide bridges are present in the native protein, they may be reoxidized using known methods.

By way of specific non-limiting example, the recombinant bacterial cells, for example *E. coli,* are grown in any of a number of suitable media, for example LB, and the expression of the recombinant protein induced by adding IPTG (*e.g.*, *lac* operator-promoter) to the media or switching incubation to a higher temperature (*e.g.*, λ cI^{857}). After culturing the bacteria for a further period of between 2 and 24 hours, the cells are collected by centrifugation and washed to remove residual media. The bacterial cells are then lysed, for example, by disruption in a cell homogenizer and centrifuged to separate the cell membranes from the soluble cell components. If the protein aggregates into inclusion bodies, this centrifugation can be performed under conditions whereby the dense inclusion bodies are selectively enriched by incorporation of sugars such as sucrose into the buffer and centrifugation at a selective speed. The inclusion bodies can then be washed in any of several solutions to remove some of the contaminating host proteins, then solubilized in solutions containing high concentrations of urea (*e.g.* 8M) or chaotropic agents such as guanidinium hydrochloride in the presence of reducing agents such as ß-mercaptoethanol or DTT (dithiothreitol).

At this stage it may be advantageous to incubate the protein for several hours under conditions suitable for the protein to undergo a refolding process into a conformation which

more closely resembles that of the native protein. Such conditions generally include low protein concentrations less than 500 μg/ml), low levels of reducing agent, concentrations of urea less than 2 M and often the presence of reagents such as a mixture of reduced and oxidized glutathione which facilitate the interchange of disulphide bonds within the protein molecule. The refolding process can be monitored, for example, by SDS-PAGE or with antibodies which are specific for the native molecule. Following refolding, the protein can then be purified further and separated from the refolding mixture by chromatography on any of several supports including ion exchange resins, gel permeation resins or on a variety of affinity columns.

Labeling Proteins

When used as a component in assay systems such as those described, below, the target protein may be labeled, either directly or indirectly, to facilitate detection of the present *res*-like molecules either *in vitro* or *in vivo*. Any of a variety of suitable labeling systems may be used including but not limited to radioisotopes such as ^{125}I; enzyme labeling systems that generate a detectable colorimetric signal or light when exposed to substrate; and fluorescent labels.

Where recombinant DNA technology is used for protein production the, it may be advantageous to engineer fusion proteins that can facilitate labeling, immobilization and/or detection. These fusion proteins may, for example, add amino acids which facilitate further chemical modification. They also may add a functional moiety, such as an enzyme, which directly facilitates detection.

TRANSGENIC ANIMALS

The invention further contemplates animal models for studying the function of the present molecules and for overproducing the protein products. The disclosed DNA sequences may be used in conjunction with techniques for producing transgenic animals that are well known to those of skill in the art.

To prepare transgenic animals, target gene sequences may for example be introduced into, and overexpressed in, the genome of the animal of interest, or, if endogenous target gene sequences are present, they may either be overexpressed or, alternatively, be disrupted in order to underexpress or inactivate target gene expression, such as described for the disruption of apoE in mice (Plum *et al.*, *Cell* 71: 343-353 (1992)).

In order to overexpress a target gene sequence, the coding portion of the target gene sequence may be ligated to a regulatory sequence which is capable of driving gene expression in the animal and cell type of interest. Such regulatory regions will be well known to those of skill in the art, and may be utilized in the absence of undue experimentation.

For underexpression of an endogenous target gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the animal of interest, the endogenous target gene alleles will be inactivated. Preferably, the engineered target gene sequence is introduced via gene targeting such that the endogenous target sequence is disrupted upon integration of the engineered target gene sequence into the animal's genome.

Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate cardiovascular disease animal models. Goats, cows and sheep are particularly preferred for producing protein *in vivo*.

Any technique known in the art may be used to introduce a target gene transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe *et al.*, U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985)); gene targeting in embryonic stem cells (Thompson *et al.*, *Cell* 56:313-321 (1989)); electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803-1814 (1983)); and sperm-mediated gene transfer (Lavitrano *et al.*, *Cell* 57:717-723 (1989)); *etc.* For a review of such techniques, see Gordon, Transgenic Animals, *Intl. Rev. Cytol.* 115:171-229 (1989).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching

of Lasko et al. (Lasko *et al.*, *Proc. Natl. Acad. Sci.* USA 89:3232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the target gene be integrated into the chromosomal site of the endogenous target gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous target gene of interest are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous target gene.

The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene of interest in only that cell type, by following, for example, the teaching of Gu *et al. Science* 265: 103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant target gene and protein may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the target gene transgene gene product of interest.

The transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using antibodies directed against the target gene product's epitopes) at easily detectable levels should then be further evaluated to identify those animals which display characteristic increased susceptibility to carcinogenesis. Additionally, specific cell types within the transgenic animals may be analyzed and assayed *in vitro* for cellular phenotypes characteristic of mutant phenotype.

Once target gene transgenic founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include but are not limited to: outbreeding of founder animals with more

than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound target gene transgenics that express the target gene transgene of interest at higher levels because of the effects of additive expression of each target gene transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order both to augment expression and eliminate the possible need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; breeding animals to different inbred genetic backgrounds so as to examine effects of modifying alleles on expression of the target gene transgene and the possible development of carcinogenesis. One such approach is to cross the target gene transgenic founder animals with a wild type strain to produce an F1 generation that exhibits increased susceptibility to carcinogenesis. The F1 generation may then be inbred in order to develop a homozygous line, if it is found that homozygous target gene transgenic animals are viable.

Methods of generating "knockout" mice using homologous recombination in embryonic stem cells are well known in the art. Suitable methods are described, for example, in Mansour *et al.*, *Nature*, 336:348 (1988); Zijlstra *et al.*, *Nature*, 342:435 (1989) and 344:742 (1990); and Hasty *et al.*, *Nature*, 350:243 (1991). This genomic DNA can be obtained by conventional methods using the cDNA sequence as a probe in a commercially-available genomic DNA library.

Briefly, a genomic fragment is cleaved with a restriction endonuclease and a heterologous cassette containing a neomycin-resistance gene is inserted at the cleavage site. A suitable cassette is the GTI-II *neo* cassette described by Lufkin *et al.*, *Cell* 66:1105 (1991). The modified genomic fragment is cloned into a suitable targeting vector that is introduced into murine embryonic stem cells by electroporation. Cells that have undergone homologous recombination (and hence disruption of the gene) are selected by resistance to G418, and used to generate chimeric mice using well known methods. See Lufkin *et al.*, *supra*. Traditional breeding methods then can be used to generate mice that are homozygous for the disrupted gene.

The phenotype of mice that are homozygous for the mutation then can be studied to provide insights into the role of the protein in, for example, carcinogenesis. These mice also can be used as models for developing new treatments for cancers. If this mutation is lethal in

homozygous mice (for example during embryogenesis) heterozygous mice, which express only half the amount of the protein can also be studied.

GENE THERAPY APPLICATIONS

When mutations in the inventive protein, or in the elements controlling expression of that protein, are found to be associated with a malignant phenotype, control of cellular proliferation can be restored by gene therapy methods. For example, overexpression of the protein can be counteracted by concurrent expression of an antisense molecule that binds to and inhibits expression of the mRNA encoding the protein. Alternatively, overexpression can be inhibited in an analogous manner using a ribozyme that cleaves the mRNA. In another embodiment, where expression of a mutated protein induces the malignant phenotype, concomitant expression of the non-mutated molecule via introduction of an exogenous gene may be used. Methods of using antisense and ribozyme technology to control gene expression, or of gene therapy methods for expression of an exogenous gene in this manner are well known in the art.

Each of these methods requires a system for introducing a vector into the cells containing the mutated gene. The vector encodes either an antisense or ribozyme transcript of the inventive protein. The construction of a suitable vector can be achieved by any of the methods well-known in the art for the insertion of exogenous DNA into a vector. *See, e.g.*, Sambrook *et al.*, *Molecular Cloning* (Cold Spring Harbor Press 2d ed. 1989), which is incorporated herein by reference. In addition, the prior art teaches various methods of introducing exogenous genes into cells *in vivo*. *See* Rosenberg *et al.*, *Science 242*:1575-1578 (1988) and Wolff *et al.*, *PNAS* 86:9011-9014 (1989), which are incorporated herein by reference. The routes of delivery include systemic administration and administration *in situ*. Well-known techniques include systemic administration with cationic liposomes, and administration *in situ* with viral vectors. Any one of the gene delivery methodologies described in the prior art is suitable for the introduction of a recombinant vector containing an inventive gene according to the invention into a MTX-resistant, transport-deficient cancer cell. A listing of present-day vectors suitable for the purpose of this invention is set forth in Hodgson, *Bio/Technology 13*: 222 (1995), which is incorporated by reference.

For example, liposome-mediated gene transfer is a suitable method for the introduction of a recombinant vector containing an inventive gene according to the invention

into a MTX-resistant, transport-deficient cancer cell. The use of a cationic liposome, such as DC-Chol/DOPE liposome, has been widely documented as an appropriate vehicle to deliver DNA to a wide range of tissues through intravenous injection of DNA/cationic liposome complexes. *See* Caplen *et al.*, *Nature Med.* 1:39-46 (1995) and Zhu *et al.*, *Science 261*:209-211 (1993), which are herein incorporated by reference. Liposomes transfer genes to the target cells by fusing with the plasma membrane. The entry process is relatively efficient, but once inside the cell, the liposome-DNA complex has no inherent mechanism to deliver the DNA to the nucleus. As such, the most of the lipid and DNA gets shunted to cytoplasmic waste systems and destroyed. The obvious advantage of liposomes as a gene therapy vector is that liposomes contain no proteins, which thus minimizes the potential of host immune responses.

As another example, viral vector-mediated gene transfer is also a suitable method for the introduction of the vector into a target cell. Appropriate viral vectors include adenovirus vectors and adeno-associated virus vectors, retrovirus vectors and herpesvirus vectors.

Adenoviruses are linear, double stranded DNA viruses complexed with core proteins and surrounded by capsid proteins. The common serotypes 2 and 5, which are not associated with any human malignancies, are typically the base vectors. By deleting parts of the virus genome and inserting the desired gene under the control of a constitutive viral promoter, the virus becomes a replication deficient vector capable of transferring the exogenous DNA to differentiated, non-proliferating cells. To enter cells, the adenovirus fibre interacts with specific receptors on the cell surface, and the adenovirus surface proteins interact with the cell surface integrins. The virus penton-cell integrin interaction provides the signal that brings the exogenous gene-containing virus into a cytoplasmic endosome. The adenovirus breaks out of the endosome and moves to the nucleus, the viral capsid falls apart, and the exogenous DNA enters the cell nucleus where it functions, in an epichromosomal fashion, to express the exogenous gene. Detailed discussions of the use of adenoviral vectors for gene therapy can be found in Berkner, *Biotechniques 6*:616-629 (1988) and Trapnell, *Advanced Drug Delivery Rev. 12*:185-199 (1993), which are herein incorporated by reference. Adenovirus-derived vectors, particularly non-replicative adenovirus vectors, are characterized by their ability to accommodate exogenous DNA of 7.5 kB, relative stability, wide host range, low pathogenicity in man, and high titers (10^4 to 10^5 plaque forming units per cell). *See* Stratford-Perricaudet *et al.*, *PNAS 89*:2581 (1992).

Adeno-associated virus (AAV) vectors also can be used for the present invention. AAV is a linear single-stranded DNA parvovirus that is endogenous to many mammalian species. AAV has a broad host range despite the limitation that AAV is a defective parvovirus which is dependent totally on either adenovirus or herpesvirus for its reproduction *in vivo*. The use of AAV as a vector for the introduction into target cells of exogenous DNA is well-known in the art. *See, e.g.*, Lebkowski *et al.*, *Mole. & Cell. Biol.* 8:3988 (1988), which is incorporated herein by reference. In these vectors, the capsid gene of AAV is replaced by a desired DNA fragment, and transcomplementation of the deleted capsid function is used to create a recombinant virus stock. Upon infection the recombinant virus uncoats in the nucleus and integrates into the host genome.

Another suitable virus-based gene delivery mechanism is retroviral vector-mediated gene transfer. In general, retroviral vectors are well-known in the art. *See* Breakfield *et al.*, *Mole. Neuro. Biol. 1*:339 (1987) and Shih *et al.*, in *Vaccines* 85: 177 (Cold Spring Harbor Press 1985). A variety of retroviral vectors and retroviral vector-producing cell lines can be used for the present invention. Appropriate retroviral vectors include Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. These vectors include replication-competent and replication-defective retroviral vectors. In addition, amphotropic and xenotropic retroviral vectors can be used. In carrying out the invention, retroviral vectors can be introduced to a tumor directly or in the form of free retroviral vector producing-cell lines. Suitable producer cells include fibroblasts, neurons, glial cells, keratinocytes, hepatocytes, connective tissue cells, ependymal cells, chromaffin cells. *See* Wolff *et al.*, *PNAS 84*:3344 (1989).

Retroviral vectors generally are constructed such that the majority of its structural genes are deleted or replaced by exogenous DNA of interest, and such that the likelihood is reduced that viral proteins will be expressed. *See* Bender *et al.*, *J. Virol.* 61:1639 (1987) and Armento *et al.*, *J. Virol. 61*:1647 (1987), which are herein incorporated by reference. To facilitate expression of the antisense or ribozyme molecule, of the inventive protein, a retroviral vector employed in the present invention must integrate into the genome of the host cell genome, an event which occurs only in mitotically active cells. The necessity for host cell replication effectively limits retroviral gene expression to tumor cells, which are highly

replicative, and to a few normal tissues. The normal tissue cells theoretically most likely to be transduced by a retroviral vector, therefore, are the endothelial cells that line the blood vessels that supply blood to the tumor. In addition, it is also possible that a retroviral vector would integrate into white blood cells both in the tumor or in the blood circulating through the tumor.

The spread of retroviral vector to normal tissues, however, is limited. The local administration to a tumor of a retroviral vector or retroviral vector producing cells will restrict vector propagation to the local region of the tumor, minimizing transduction, integration, expression and subsequent cytotoxic effect on surrounding cells that are mitotically active.

Both replicatively deficient and replicatively competent retroviral vectors can be used in the invention, subject to their respective advantages and disadvantages. For instance, for tumors that have spread regionally, such as lung cancers, the direct injection of cell lines that produce replication-deficient vectors may not deliver the vector to a large enough area to completely eradicate the tumor, since the vector will be released only form the original producer cells and their progeny, and diffusion is limited. Similar constraints apply to the application of replication deficient vectors to tumors that grow slowly, such as human breast cancers which typically have doubling times of 30 days versus the 24 hours common among human gliomas. The much shortened survival-time of the producer cells, probably no more than 7-14 days in the absence of immunosuppression, limits to only a portion of their replicative cycle the exposure of the tumor cells to the retroviral vector.

The use of replication-defective retroviruses for treating tumors requires producer cells and is limited because each replication-defective retrovirus particle can enter only a single cell and cannot productively infect others thereafter. Because these replication-defective retroviruses cannot spread to other tumor cells, they would be unable to completely penetrate a deep, multilayered tumor *in vivo*. *See* Markert *et al., Neurosurg. 77*: 590 (1992). The injection of replication-competent retroviral vector particles or a cell line that produces a replication-competent retroviral vector virus may prove to be a more effective therapeutic because a replication competent retroviral vector will establish a productive infection that will transduce cells as long as it persists. Moreover, replicatively competent retroviral vectors may follow the tumor as it metastasizes, carried along and propagated by transduced tumor cells. The risks for complications are greater, with replicatively competent vectors, however.

Such vectors may pose a greater risk then replicatively deficient vectors of transducing normal tissues, for instance. The risks of undesired vector propagation for each type of cancer and affected body area can be weighed against the advantages in the situation of replicatively competent verses replicatively deficient retroviral vector to determine an optimum treatment.

Both amphotropic and xenotropic retroviral vectors may be used in the invention. Amphotropic viruses have a very broad host range that includes most or all mammalian cells, as is well known to the art. Xenotropic viruses can infect all mammalian cell cells except mouse cells. Thus, amphotropic and xenotropic retroviruses from many species, including cows, sheep, pigs, dogs, cats, rats, and mice, *inter alia* can be used to provide retroviral vectors in accordance with the invention, provided the vectors can transfer genes into proliferating human cells *in vivo*.

Clinical trials employing retroviral vector therapy treatment of cancer have been approved in the United States. *See* Culver, *Clin. Chem. 40*: 510 (1994). Retroviral vector-containing cells have been implanted into brain tumors growing in human patients. *See* Oldfield *et al., Hum. Gene Ther. 4*: 39 (1993). These retroviral vectors carried the HSV-1 thymidine kinase (HSV-tk) gene into the surrounding brain tumor cells, which conferred sensitivity of the tumor cells to the antiviral drug ganciclovir. Some of the limitations of current retroviral based cancer therapy, as described by Oldfield are: (1) the low titer of virus produced, (2) virus spread is limited to the region surrounding the producer cell implant, (3) possible immune response to the producer cell line, (4) possible insertional mutagenesis and transformation of retroviral infected cells, (5) only a single treatment regimen of pro-drug, ganciclovir, is possible because the "suicide" product kills retrovirally infected cells and producer cells and (6) the bystander effect is limited to cells in direct contact with retrovirally transformed cells. *See* Bi *et al., Human Gene Therapy 4*: 725 (1993).

Yet another suitable virus-based gene delivery mechanism is herpesvirus vector-mediated gene transfer. While much less is known about the use of herpesvirus vectors, replication-competent HSV-1 viral vectors have been described in the context of antitumor therapy. *See* Martuza *et al., Science 252*: 854 (1991), which is incorporated herein by reference.

DIAGNOSTIC METHODS

The present invention also contemplates, for certain molecules described below, methods for diagnosis of human disease. In particular, patients can be screened for the occurrence of cancers, or likelihood of occurrence of cancers, associated with mutations in the encoded protein. DNA from tumor tissue obtained from patients suffering from cancer can be isolated and the gene encoding the protein can be sequenced. By examining a number of patients in this manner, mutations in the gene that are associated with a malignant cellular phenotype can be identified. In addition, correlation of the nature of the observed mutations with subsequent observed clinical outcomes allows development of prognostic model for the predicted outcome in a particular patient.

Screening for mutations conveniently can be carried out at the DNA level by use of PCR, although the skilled artisan will be aware that many other well known methods are available for the screening. PCR primers can be selected that flank known mutation sites, and the PCR products can be sequenced to detect the occurrence of the mutation. Alternatively, the 3' residue of one PCR primer can be selected to be a match only for the residue found in the unmutated gene. If the gene is mutated, there will be a mismatch at the 3' end of the primer, and primer extension cannot occur, and no PCR product will be obtained. Alternatively, primer mixtures can be used where the 3' residue of one primer is any nucleotide other than the nonmutated residue. Observation of a PCR product then indicates that a mutation has occurred. Other methods of using, for example, oligonucleotide probes to screen for mutations are described, or example, in U.S. Patent No. 4,871,838, which is herein incorporated by reference in its entirety.

Alternatively, antibodies can be generated that selectively bind either mutated or non-mutated protein. The antibodies then can be used to screen tissue samples for occurrence of mutations in a manner analogous to the DNA-based methods described *supra*.

The diagnostic methods described above can be used not only for diagnosis and for prognosis of existing disease, but may also be used to predict the likelihood of the future occurrence of disease. For example, clinically healthy patients can be screened for mutations in the inventive molecule that correlate with later disease onset. Such mutations may be observed in the heterozygous state in healthy individuals. In such cases a single mutation event can effectively disable proper functioning of the gene and induce a transformed or malignant phenotype. This screening also may be carried out prenatally or neonatally.

DNA molecules according to the invention also are well suited for use in so-called "gene chip" diagnostic applications. Such applications have been developed by, *inter alia*, Synteni and Affymetrix. Briefly, all or part of the DNA molecules of the invention can be used either as a probe to screen a polynucleotide array on a "gene chip," or they may be immobilized on the chip itself and used to identify other polynucleotides via hybridization to the surface of the chip. In this manner, for example, related genes can be identified, or expression patterns of the gene in various tissues can be simultaneously studied. Such gene chips have particular application for diagnosis of disease, or in forensic analysis to detect the presence or absence of an analyte. Suitable chip technology is described for example, in Wodicka *et al.*, *Nature Biotechnology*, 15:1359 (1997) which is hereby incorporated by reference in its entirety, and references cited therein.

PROTEIN-PROTEIN INTERACTIONS

Due to their similarity to certain known proteins, it is anticipated that some of the inventive protein molecules will interact with another class of cellular proteins. This is particularly true of those molecule containing leucine zipper motifs.

Any method suitable for detecting protein-protein interactions can be employed for identifying interacting targets. Among the traditional methods which can be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns. Utilizing procedures such as these allows for the identification of GAP gene products. Once identified, a GAP protein can be used, in conjunction with standard techniques, to identify its corresponding pathway gene. For example, at least a portion of the amino acid sequence of the pathway gene product can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique (see, e.g., Creighton, 1983, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained can be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for pathway gene sequences. Screening can be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and for screening are well-known. (See *e.g.*, Ausubel, *supra*, and PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, 1990, Innis *et al.*, eds. Academic Press, Inc., New York).

Additionally, methods can be employed which result in the simultaneous identification of interacting target genes. One method which detects protein interactions *in vivo*, the two-hybrid system, is described in detail for illustration purposes only and not by way of limitation. One version of this system has been described (Chien *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 9578-9582 (1991)) and is commercially available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to a known protein, in this case an inventive protein, and the other contains the activator protein's activation domain fused to an unknown protein (a putative GAP, for instance) that is encoded by a cDNA which has been recombined into this plasmid as part of a cDNA library. The plasmids are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (*e.g.*, *lacZ*) whose regulatory region contains the transcription activator's binding sites. Either hybrid protein alone cannot activate transcription of the reporter gene, the DNA-binding domain hybrid cannot because it does not provide activation function, and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology can be used to screen activation domain libraries for proteins that interact with a known "bait" gene product. By way of example, and not by way of limitation, gene products known to be involved in TH cell subpopulation-related disorders and/or differentiation, maintenance, and/or effector function of the subpopulations can be used as the bait gene products. Total genomic or cDNA sequences are fused to the DNA encoding on activation domain. This library and a plasmid encoding a hybrid of the bait gene product fused to the DNA-binding domain are cotransformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, and not by way of limitation, the bait gene can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

The present invention, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

EXAMPLES

EXAMPLE I: cDNA Library Construction

cDNA library plates and clones originated from five cDNA libraries that were constructed by directional cloning. These are available through the Resource Center (http://www.rzpd.de) of the German Genome Project. In particular, the hfbr2 (human fetal brain; RZPD number DKFZp564) and hfkd2 (human fetal kidney; DKFZp566) libraries were generated using the Smart kit (Clontech), except that PCR was carried out with primers that contained uracil residues to permit directional cloning without restriction digestion and ligation, and were complementary with the pAMP1 (LifeTechnologies) cloning sites for directional cloning. The htes3 (human testes; DKFZp434), hutel (human uterus; DKFZp586) and hmcf1 (human mammary carcinoma; DKFZp727) libraries are conventional (Gubler, U., Hoffman, B.J., (1983), A simple and very efficient method for generating cDNA libraries. Gene 25, 263-269), size-selected cDNA libraries. They are cloned into pSPORT1 (LifeTechnologies) via a NotI site which is introduced during reverse transcription downstream of the oligo dT primer and a SalI site that is introduced by the ligation of a adapters. The human mammary carcinoma library was constructed fgrom MCF7 cells.

The cDNA sequences of this application were first identified among the sequences comprising various libraries. Technology has advanced considerably since the first cDNA libraries were made. Many small variations in both chemicals and machinery have been instituted over time, and these have improved both the efficiency and safety of the process. Although the cDNAs could be obtained using an older procedure, the procedure presented in this application is exemplary of one currently being used by persons skilled in the art. For the

purpose of providing an exemplary method, the mRNA isolation and cDNA library construction described here is for the MCF-7 library (DKFZp727) from which the clones named DKFZphmcf1_xxyyxx were obtained.

The human cell line MCF-7 was grown in DMEM supplemented with 10% fetal calf serum until confluency. 3 X 10^8 cells were harvested with a cell scraper in PBS. Cells were lysed in buffer containing 0.5 % NP-40 to leave the nuclei intact. The debris was pelleted by centrifugation at 15 000 x g for 10 minutes at 4 degrees Celsius. Proteins in the supernatant were degraded in presence of SDS and Proteinase K (30 minutes at 56 degrees Celsius). Precipitation of proteins was done in a Phenol/Chloroform extraction, RNA was precipitated from the aqueous phase with Na-acetate and Ethanol. Polyadenylated messages were isolated using Qiagen Oligotex (QIAGEN, Hilden Germany).

First strand cDNA synthesis was accomplished using an oligo (dT) primer which also contained an NotI restriction site. Second strand synthesis was performed using a combination of DNA polymerase I, *E. coli* ligase and RNase H, followed by the addition of a SalI adaptor to the blunt ended cDNA. The SalI adapted, double-stranded cDNA was then digested with NotI restriction enzyme, and fractionated by size on an agarose gel. DNA of the appropriate size was cut from the gel and cast into a second gel in a 90° angle. After electrophoresis in the second dimension, cDNA of the appropriate size was cut from the gel. The agarose block was broken down with help of gelase. The cDNA was purified with help of two phenol extractions and an ethanol precipitation. The cDNA was ligated into SalI/NotI pre-digested pSport1 vector (LifeTechnologies) and transformed into DH10B bacteria.

The libraries were arrayed into 384-well microtiter plates and spotted on high density nylon membranes for hybridization analysis. Filters and clones are available through the Resource Center. Whole plates were distributed to the sequencing partners of the consortium for systematic sequencing.

EXAMPLE II: Sequencing of cDNA Clones

All clones in the 384-well microtiter plates were sequenced from the 5' end. Sequencing was done preferentially using dye terminator chemistry (ABD or Amersham) on

ABI automated DNA sequencers (ABI 377, Applied Biosystems), one partner used EMBL prototype instruments (Arakis) mainly with dye primer chemistry.

The resulting expressed sequence tag (EST) sequences ("r1 ESTs" = sequenced from 5'-end) were analysed for:

a) the lack of identical matches with known genes.

For this, the EST-sequence was blasted against the cDNA consortiums own database and after that against public databases and (with BLASTn and BLASTx against EMBL/EMBLNEW and assembled ESTs, please refer to EXAMPLE III: Bioinformatics analysis of full length cDNAs, for description and parameter settings). ESTs which were identical to known genes in more than 100 bp, with less than 2 mismatches, were excluded from further analysis.

b) the presence of an open reading frame

Open reading frames (ORFs) were detected with an tool developed by Munich Information Center for Protein Sequences (MIPS) called ORF-map. ORF-map visualises potential start and stop-codons. If an ORF without a stop codon was detected in a r1-EST, the sequence was processed further.

c) the presence of GC rich sequences

A script developed by MIPS computed the GC-content of the r1-sequence, which should be >40%. Writing similar scripts is within the ordinary skill of one in bioinformatics.

d) the lack of repeat structures

Repeats such as Alu, Line or CA-repeats were detected by blasting (BLASTn and BLASTx, please refer to EXAMPLE III: Bioinformatics analysis of full length cDNAs, for description and parameter settings) against a repeat-database compiled by MIPS. If a repeat was present within the r1-sequence, the sequence were not processed further.

Novel clones that met all criteria were identified to the sequencers, who then performed 3'-end sequencing of these clones. The resulting 3' ESTs ("s1 ESTs" = sequenced from 3'-end) were checked for

a) the lack of matches with known genes in public databases, and sequences already generated by us.

This was done by blasting against EMBL/EMBLNEW and assembled EST (BLASTn and BLASTx, please refer to EXAMPLE III: Bioinformatics analysis of full length cDNAs, for description and parameter settings).

b) the presence of polyadenylation signals.

Again only clones matching the selection criteria were chosen to be sequenced completely by the sequencers. Clones were selected after the following criteria:

A very good ORF had at least one BLASTx match to other proteins. A "good ORF" should extend to the 3' end and be longer than ~40 codons. If the ORF started in the r1 sequence, in front of the potential start codon, there should not exist too many competing start codons in frame with the ORF start codon and the start should match the Kozak consensus ATG. If the EST sequence was to short to decide according to the potential ORF, and there were only a few or no start codons in the sequence the GC content of the Sequence should be greater than 40%. The r1 sequences needed not contain an polyA-tail at the 3' end. In addition, the results of the blasting against the assembled human ESTs could help in questionable cases to decide whether to stop or to continue. A hit against these ESTs was an indication to go further.

Clones passing the above-described screening were sequenced in full. Sequencing was done preferentially using dye terminator chemistry (ABD or Amersham) on ABI automated DNA sequencers (ABI 377, Applied Biosystems), one partner used EMBL prototype instruments (Arakis) mainly with dye primer chemistry. Primer walking (Strauss et al., 1986, Specific-primer-directed DNA sequencing. Anal Biochem. 154, 353-360) was the preferred sequencing strategy because of the lower redundancy possible compared to random shotgun (Messing, J., Crea, R., Seeburg, H.P. (1981) A system for shotgun DNA sequencing. Nucleic Acids Res. 9, 32-39) methods. Walking primers were generally designed using software (e.g. Haas, S., Vingron, M., Poustka, A., Wiemann, S. (1998) Primer design in large-scale sequencing. Nucleic Acids Res. 26, 3006-3012, Schwager, C., Wiemann, S., Ansorge, W. (1995) GeneSkipper: integrated software environment for DNA sequence assembly and

alignment. HUGO Genome Digest 2, 8-9) that permitted complete automation of this usually time consuming process and helped in the parallel processing of large numbers of clones.

EXAMPLE III: Bioinformatics analysis of full length cDNAs

Each sequence obtained was compared on nucleotide level in a stepwise manner to sequences in EMBL/EMBLNEW, EMBL-EST, EMBL-STS using the BLASTn algorithm. Basic Local Alignment Search Tool (BLAST, Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S. F. et al (1990) J Mol Biol 215:403-10) is used to search for local sequence alignments. BLAST produces alignments of both nucleotide (BLASTn) and amino acid sequences (BLASTp or BLASTx) to determine sequence similarity. BLAST is especially useful in determining exact matches or in identifying homologs, because of the local nature of the alignments. While it is useful for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment BLAST approach is to look threshold or cut off score set by the user. BLAST looks for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output. Parameter settings for the BLAST-operations (BLASTN 2.0a19MP-WashU) described were: EMBL-EMBLNEW: H=0 V=5 B=5 -filter seg; EMBL-EST: H=0 E=1e-10 B=500 V=500 -filter seg; EMBL-STS: H=0 V=5 B=5.

Search against EMBL/EMBLNEW was done to determine whether the cDNAs are already known, and also to find out whether the cDNAs are encoded by genomic sequences already sequenced and published/submitted to these databases.

Search against EMBL-EST was performed to get a first impression how abundant a particular cDNA would be and to get information on tissue specificity (so-called "electronic Northern-Blot", e.g. some of the cDNAs derived of the testis library show only hits to ESTs also derived of testis libraries).

The cDNA-sequences were blasted against EMBL-STS to determine STS-sequence-match to the cDNA, thus providing a mapping information to the new cDNA.

The potential protein-sequences were generated automatically by a script searching for the longest open reading frame (ORF) in each of the three forward frames with a minimum length of 90 codons. Next, the automatically generated ORFs were translated into protein sequences. These protein sequences were searched against the non redundant protein data set of PIR/SwissProt/Trembel/Tremblnew (BLASTP 2.0a19MP-WashU, parameter setting: V=7 B=7 H=0 -filter seg). If the script generated more than one ORF, one ORF was chosen manually by the annotater according to the degree of similarity to known proteins, the location of the ORF in the cDNA, the length, the amino acid composition and the content of Prosite-Motifs.

Additionally there was a BLASTx (BLASTX 2.0a19MP-WashU against non redundant protein database comprising PIR/SWISSPROT/TREMBL/TREMBLNEW; parameter-settings were: matrix/home/data/blast/matrix/aa/BLOSUM62 H=0 V=5 B=5 -filter seg) search to find potential frame shift in the complementary cds of the cDNAs and to identify unspliced or partly spliced cDNAs. The protein sequence was then transferred to the PEDANT system, in order to generate additional information on the new proteins. PEDANT (Protein Extraction, Description, and ANalysis Tool, Frishman, D. & Mewes, H.-W. (1997) PEDANTic genome analysis. Trends in Genetics , 13, 415-416) is a platform developed at the Munich Information Center for Protein Sequences (MIPS, Munich, Germany), which incorporates practically all bioinformatics methods important for the functional and structural characterisation of protein sequences. Computational methods used by PEDANT are:

FASTA

Very sensitive protein sequence database searches with estimates of statistical significance. Pearson W.R. (1990) Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol. 183, 63-98.

BLAST2

Very sensitive protein sequence database searches with estimates of statistical significance. Altschul S.F., Gish W., Miller W., Myers E.W., and Lipman D.J. Basic local alignment search tool. Journal of Molecular Biology 215, 403-10.

PREDATOR

High-accuracy secondary structure prediction from single and multiple sequences. Frishman, D. and Argos, P. (1997) 75% accuracy in protein secondary structure prediction. Proteins, 27, 329-335. Frishman, D. and Argos, P.(1996) Incorporation of long-distance interactions in a secondary structure prediction algorithm. Prot. Eng. 9, 133-142.

STRIDE

Secondary structure assignment from atomic coordinates. Frishman, D. and Argos, P.(1995) Knowledge-based secondary structure assignment. Proteins 23, 566-579.

CLUSTALW

Multiple sequence alignment. Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680.

TMAP

Transmembrane region prediction from multiply aligned sequences. Persson, B. and Argos, P. (1994) Prediction of transmembrane segments in proteins utilising multiple sequence alignments. J. Mol. Biol. 237, 182-192.

ALOM2

Transmembrane region prediction from single sequences. Klein, P., Kanehisa, M., and DeLisi, C. Prediction of protein function from sequence properties: A discriminant analysis of a database. Biochim. Biophys. Acta 787, 221-226 (1984). Version 2 by Dr. K. Nakai.

SIGNALP

Signal peptide prediction Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G (1997). Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Engineering 10, 1-6.

SEG

Detection of low complexity regions in protein sequences. Wootton, J.C., Federhen, S. (1993) Statistics of local complexity in amino acid sequences and sequence databases. Computers & Chemistry 17, 149-163.

COILS

Detection of coiled coils. Lupas, A., M. Van Dyke, and J. Stock, "Predicting Coiled Coils from Protein Sequences." Science (1991) 252, 1162-1164.

PROSEARCH

Detection of PROSITE protein sequence patterns. Kolakowski L.F. Jr., Leunissen J.A.M., Smith J.E. (1992) ProSearch: fast searching of protein sequences with regular expression patterns related to protein structure and function. Biotechniques 13, 919-921.

BLIMPS

Similarity searches against a database of ungapped blocks. J.C. Wallace and Henikoff S., (1992) PATMAT: a searching and extraction program for sequence, pattern and block queries and databases, CABIOS 8, 249-254. Written by Bill Alford.

HMMER

Hidden Markov model software . Sonnhammer E.L.L., Eddy S.R., Durbin R. (1997) Pfam: A Comprehensive Database of Protein Families Based on Seed Alignments. Proteins 28, 405-420.

pI

Perl script that returns the amino acid composition, molecular weight, theoretical pI, and expected extinction coefficient of an amino acid sequence. By Fred Lindberg. The parameter-settings were as follows: known3d: score > 100; BLAST: E-value < 10; SCOP: <= 50 Alignments, E-Value < 0.0001; signalp: Y=0.7; untersucht vom N-Terminus her: 50 aa; funcat: E-value < 0.001; BLOCKS: <= 10 hits; BLIMPS: threshold 1100.0; COILS: threshold 0.95; SEG: threshold 20.0; BLAST in report: E-value < 0.001; PIR-KW, superfamilies, EC-Nummern in report: E-value < 0.00001; known3d in report: score > 120

The results of PEDANT analysis, together with the results of the similarity searches, constitute the basis for the structural and functional annotation of the cDNAs and the encoded proteins, as specified below.

EXAMPLE III: CELLULAR LOCALIZATIONS OF GFP-FUSION PROTEINS

Plasmids of cDNA-GFP fusions were transfected into mammalian tissue culture cells and allowed to express the proteins for up to 48 hours. Live cells were imaged at 24 hours and 48 hours after transfection and the localisations recorded. The chart, below, depicts the apparent final cellular localisations of 107 cDNA-GFP fusions.

In order to minimize the possibility of the GFP interfering with protein function and/or localization, two separate populations of cDNAs were generated encoding N-terminal or C-terminal GFP fusions. Clearly this appears to be a crucial strategy, since overall only 56% of the proteins localised to a specific compartment irrespective of the position of the GFP. In the instances where only one fusion localized, the complementary fusion either gave no expression or a nuclear and cytosolic staining - characteristic for GFP alone expression.

Each cDNA in turn was subjected to bioinformatic analysis. Where possible, the potential subcellular localisations of the expressed proteins were determined. This

information was then compared to the actual localisations determined from expression of the GFP-fusion proteins in mammalian cells.

DKFZphtes3_7j3
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group: cell cycle

DKFZphtes3_7j3.2 encodes a novel 628 amino acid putative protein kinase, which is related to the C-TAK1 Cdc25C associated protein kinase.

Cdc25C is a protein kinase that controls entry into mitosis by dephosphorylation of Cdc2. Cdc25C function is regulated by phosphorylation, too. Serine 216 phosphorylation of Cdc25C mediates the binding of 14-3-3 protein to Cdc25C. C-TAK1 (Cdc twenty-five C associated protein kinase) phosphorylates Cdc25C on serine 216 in vitro. The new protein is closely related to C-Tak1 and therefore should be involved in cell-cycle regulation, too.

The new protein can find application in modulating/blocking the cell cycle.

strong similarity to serine/threonine-specific protein kinases

complete cDNA, complete cds, potential start at Bp 128, few EST hits

Sequenced by BMFZ

Locus: unknown

Insert length: 3443 bp
Poly A stretch at pos. 3399, polyadenylation signal at pos. 3376

```
   1 GTGCTTTACT GCGCGCTCTG GTACTGCTGT GGCTCCCCGT CCTGGTGCGG
  51 GACCTGTGCC CCGCGCTTCA GCCCTCCCCG CACAGCCTAC TGATTCCCCT
 101 GCCGCCCTTG CTCACCTCCT GCTCGCCATG GAGTCGCTGG TTTTCGCGCG
 151 GCGCTCCGGC CCCACTCCCT CGGCCGCAGA GCTAGCCCGG CCGCTGGCGG
 201 AAGGGCTGAT CAAGTCGCCC AAGCCCCTAA TGAAGAAGCA GGCGGTGAAG
 251 CGGCACCACC ACAAGCACAA CCTGCGGCAC CGCTACGAGT TCCTGGAGAC
 301 CCTGGGCAAA GGCACCTACG GGAAGGTGAA GAAGGCGCGG GAGAGCTCGG
 351 GGCGCCTGGT GGCCATCAAG TCAATCCGGA AGGACAAAAT CAAAGATGAG
 401 CAAGATCTGA TGCACATACG GAGGGAGATT GAGATCATGT CATCACTCAA
 451 CCACCCTCAC ATCATTGCCA TCCATGAAGT GTTTGAGAAC AGCAGCAAGA
 501 TCGTGATCGT CATGGAGTAT GCCAGCCGGG GCGACCTTTA TGACTACATC
 551 AGCGAGCGGC AGCAGCTCAG TGAGCGCGAA GCTAGGCATT TCTTCCGGCA
 601 GATCGTCTCT GCCGTGCACT ATTGCCATCA GAACAGAGTT GTCCACCGAG
 651 ATCTCAAGCT GGAGAACATC CTCTTGGATG CCAATGGGAA TATCAAGATT
 701 GCTGACTTCG GCCTCTCCAA CCTCTACCAT CAAGGCAAGT TCCTGCAGAC
 751 ATTCTGTGGG AGCCCCCTCT ATGCCTCGCC AGAGATTGTC AATGGGAAGC
 801 CCTACACAGG CCCAGAGGTG GACAGCTGGT CCCTGGGTGT TCTCCTCTAC
 851 ATCCTGGTGC ATGGCACCAT GCCCTTTGAT GGGCATGACC ATAAGATCCT
 901 AGTGAAACAG ATCAGCAACG GGGCCTACCG GGAGCCACCT AAACCCTCTG
 951 ATGCCTGTGG CCTGATCCGG TGGCTGTTGA TGGTGAACCC CACCCGCCGG
1001 GCCACCCTGG AGGATGTGGC CAGTCACTGG TGGGTCAACT GGGGCTACGC
1051 CACCCGAGTG GGAGAGCAGG AGGCTCCGCA TGAGGGTGGG CACCCTGGCA
1101 GTGACTCTGC CCGCGCCTCC ATGGCTGACT GGCTCCGGCG TTCCTCCCGC
1151 CCCCTCCTGG AGAATGGGGC CAAGGTGTGC AGCTTCTTCA AGCAGCATGC
1201 ACCTGGTGGG GGAAGCACCA CCCCTGGCCT GGAGCGCCAG CATTCGCTCA
1251 AGAAGTCCCG CAAGGAGAAT GACATGGCCC AGTCTCTCCA CAGTGACACG
1301 GCTGATGACA CTGCCCATCG CCCTGGCAAG AGCAACCTCA AGCTGCCAAA
1351 GGGCATTCTC AAGAAGAAGG TGTCAGCCTC TGCAGAAGGG GTACAGGAGG
1401 ACCCTCCGGA GCTCAGCCCA ATCCCTGCGA GCCCAGGGCA GGCTGCCCCG
1451 CTGCTCCCCA AGAAGGGCAT TCTCAAGAAG CCCCGACAGC GCGAGTCTGG
1501 CTACTACTCC TCTCCCGAGC CCAGTGAATC TGGGGAGCTC TTGGACGCAG
1551 GCGACGTGTT TGTGAGTGGG GATCCCAAGG AGCAGAAGCC TCCGCAAGCT
1601 TCAGGGCTGC TCCTCCATCG CAAAGGCATC CTCAAACTCA ATGGCAAGTT
1651 CTCCCAGACA GCCTTGGAGC TCGCGGCCCC CACCACCTTC GGCTCCCTGG
1701 ATGAACTCGC CCCACCTCGC CCCCTGGCCC GGGCCAGCCG ACCCTCAGGG
1751 GCTGTGAGCG AGGACAGCAT CCTGTCCTCT GAGTCCTTTG ACCAGCTGGA
1801 CTTGCCTGAA CGGCTCCCAG AGCCCCCACT GCGGGGCTGT GTGTCTGTGG
1851 ACAACCTCAC GGGGCTTGAG GAGCCCCCCT CAGAGGGCCC TGGAAGCTGC
1901 CTGAGGCGCT GGCGGCAGGA TCCTTTGGGG GACAGCTGCT TTTCCCTGAC
1951 AGACTGCCAG GAGGTGACAG CGACCTACCG ACAGGCACTG AGGGTCTGCT
2001 CAAAGCTCAC CTGAGTGGAG TAGGCATTGC CCCAGCCCGG TCAGGCTCTC
2051 AGATGCAGCT GGTTGCACCC CGAGGGGAGA TGCCTTCTCC CCCACCTCCC
2101 AGGACCTGCA TCCCAGCTCA GAAGGCTGAG AGGGTTTGCA GTGGAGCCCT
2151 GAGCAGGGCT GGATATGGGA AGTAGGCAAA TGAAATGCGC CAAGGGTTCA
2201 GTGTCTGTCT TCAGCCCTGC TGAACGAAGA GGATACTAAA GAGAGGGGAA
2251 CGGGAATGCC CGCGACAGAG TCCACATTGC CTGTTTCTTG TGTACATGGG
2301 GGGGCCACAG AGACCTGGAA AGAGAACTCT CCCAGGGCCC ATCTCCTGCA
2351 TCCCATGAAT ACTCTGTACA CATGGTGCCT TCTAAGGACA GCTCCTTCCC
2401 TACTCATTCC CTGCCCAAGT GGGGCCAGAC CTCTTTACAC ACACATTCCC
2451 GTTCCTACCA ACCACCAGAA CTGGATGGTG GCACCCCTAA TGTGCATGAG
2501 GCATCCTGGG AATGGTCTGG AGTAACGCTT CGTTATTTTT ATTTTTATTT
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2551 TTATTTATTT ATTTATTTTT TTGAGACGGA GTTTCGCTCT TGGTGCCCAG
2601 GCTAGAGTGC AATGGCGCGA TCTCAGCTCA CCTCAACCTC CGCCTCCCGG
2651 GTTCAAGCGA TTCTCCTGCC TCAGCCTCCC TAGTAGCTGG GATTACAGGC
2701 GCCCGCCACC ATGCCCGGCT AATTTTGTAT TTTTAGTAGA GACAGGGTTT
2751 CTCCATGTTG GTCAGGCTGG TCTCAAACTC CCGACCTCAG GTGATCCACC
2801 CACCTCGGCC TCCCAAAGTG CTGGGATTAC AGGCGTGAGC CACCGCGCCC
2851 CACCTAACCC TTCCTTATTT AGCCTAGGAG TAAGAGAACA CAATCTCTGT
2901 TTCTTCAATG GTTCTCTTCC CTTTTCCATC CTCCAAACCT GGCCTGAGCC
2951 TCCTGAAGTT GCTGCTGTGA ATCTGAAAGA CTTGAAAAGC CTCCGCCTGC
3001 TGTGTGGACT TCATCTCAAG GGGCCCAGCC TCCTCTGGAC TCCACCTTGG
3051 ACCTCAGTGA CTCAGAACTT CTGCCTCTAA GCTGCTCTAA AGTCCAGACT
3101 ATGGATGTGT TCTCTAGGCC TTCAGGACTC TAGAATGTCC ATATTTATTT
3151 TTATGTTCTT GGCTTTGTGT TTTAGGAAAA GTGAATCTTG CTGTTTTCAA
3201 TAATGTGAAT GCTATGTTCT GGGAAAATCC ACTATGACAT CTAAGTTTTG
3251 TGTACAGAGA GATATTTTTG CAACTATTTC CACCTCCTCC CACAACCCCC
3301 CACACTCCAC TCCACACTCT TGAGTCTCTT TACCTAATGG TCTCTACCTA
3351 ATGGACCTCC GTGGCCAAAA AGTACCATTA AAACCAGAAA GGTGATTGGA
3401 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
```

BLAST Results

No BLAST result

Medline entries

98202387:
C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3
protein binding.

Peptide information for frame 2

ORF from 128 bp to 2011 bp; peptide length: 628
Category: strong similarity to known protein

```
   1 MESLVFARRS GPTPSAAELA RPLAEGLIKS PKPLMKKQAV KRHHHKHNLR
  51 HRYEFLETLG KGTYGKVKKA RESSGRLVAI KSIRKDKIKD EQDLMHIRRE
 101 IEIMSSLNHP HIIAIHEVFE NSSKIVIVME YASRGDLYDY ISERQQLSER
 151 EARHFFRQIV SAVHYCHQNR VVHRDLKLEN ILLDANGNIK IADFGLSNLY
 201 HQGKFLQTFC GSPLYASPEI VNGKPYTGPE VDSWSLGVLL YILVHGTMPF
 251 DGHDHKILVK QISNGAYREP PKPSDACGLI RWLLMVNPTR RATLEDVASH
 301 WWVNWGYATR VGEQEAPHEG GHPGSDSARA SMADWLRRSS RPLLENGAKV
 351 CSFFKQHAPG GGSTTPGLER QHSLKKSRKE NDMAQSLHSD TADDTAHRPG
 401 KSNLKLPKGI LKKKVSASAE GVQEDPPELS PIPASPGQAA PLLPKKGILK
 451 KPRQRESGYY SSPEPSESGE LLDAGDVFVS GDPKEQKPPQ ASGLLLHRKG
 501 ILKLNGKFSQ TALELAAPTT FGSLDELAPP RPLARASRPS GAVSEDSILS
 551 SESFDQLDLP ERLPEPPLRG CVSVDNLTGL EEPPSEGPGS CLRRWRQDPL
 601 GDSCFSLTDC QEVTATYRQA LRVCSKLT
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BLASTP hits

No BLASTP hits available

Alert BLASTP hits for DKFZphtes3_7j3, frame 2

No Alert BLASTP hits found

Pedant information for DKFZphtes3_7j3, frame 2

Report for DKFZphtes3_7j3.2

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[LENGTH]        628
[MW]            69612.39
[pI]            9.01
[HOMOL]         TREMBL:AB011109_1 gene: "KIAA0537"; product: "KIAA0537 protein"; Homo sapiens
mRNA for KIAA0537 protein, complete cds. 1e-152
[FUNCAT]        01.05.04 regulation of carbohydrate utilization      [S. cerevisiae, YDR477w]
5e-66
[FUNCAT]        11.01 stress response [S. cerevisiae, YDR477w] 5e-66
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We claim:

1. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_16f21; hfbr2_16g18; hfbr2_16i12; hfbr2_16k22; hfbr2_16l12; hfbr2_22f21; hfbr2_22h13; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23b10; hfbr2_23b21; hfbr2_23f2; hfbr2_23l24; hfbr2_23n16; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2b17; hfbr2_2b5; hfbr2_2c1; hfbr2_2c17; hfbr2_2c18; hfbr2_2d15; hfbr2_2d17; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2i17; hfbr2_2k14; hfbr2_2k19; hfbr2_3b16; hfbr2_3c18; hfbr2_3f16; hfbr2_3g8; hfbr2_3l2; hfbr2_41m15; hfbr2_62b11; hfbr2_62f10; hfbr2_62l19; hfbr2_62n10; hfbr2_62o17; hfbr2_64a11; hfbr2_64a15; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64j18; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6b24; hfbr2_6i20; hfbr2_6o17; hfbr2_71o20; hfbr2_72b18; hfbr2_72d13; hfbr2_72l12; hfbr2_72m16; hfbr2_72n12; hfbr2_78c24; hfbr2_78d13; hfbr2_78k24; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82c20; hfbr1_10c20; hfbr2_82e17; hfbr1_10e17; hfbr2_82e4;; hfbr1_10e4; hfbr2_82g14;; hfbr1_10g14; hfbr2_82i17;; hfbr1_10; hfbr2_82i24;; hfbr1_10; hfbr2_82m16;; hfbr1_10; hfbr2_82m6;; hfbr1_10; hfkd2_1j9; hfkd2_24a15; hfkd2_24b15; hfkd2_24e23; hfkd2_24n20; hfkd2_24p5; hfkd2_3i13; hfkd2_3o17; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_46j20; hfkd2_46k19; hfkd2_46m4; hfkd2_47a4; hfkd2_4b6; hfkd2_4c8; hfkd2_4k14; hfkd2_4m11; hmcf1_1a11; hmcf1_1c23; hmcf1_1e15; hmcf1_1g13; hhtes3_1n3; htes3_14g5; htes3_14h21; htes3_14p14; htes3_14p7; htes3_15a13; Htes3_15c24; htes3_15c6; htes3_15g14; htes3_15h1; htes3_15i5; htes3_15j18; Htes3_15j3; htes3_15k11; htes3_17f10; htes3_17l17; htes3_17n12; htes3_17n18; Htes3_18f3; htes3_18l7; htes3_19f19; htes3_19j17; htes3_1c1; htes3_1g13; htes3_1k11; htes3_20c21; htes3_20k2; htes3_20m18; htes3_21d4; htes3_21j15; htes3_21l16; htes3_21n23; htes3_22c23; htes3_22g2; htes3_22n13; htes3_23l11; htes3_23n19; Htes3_23n19; htes3_26g22; htes3_27d1; htes3_27k4; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2a17; htes3_2d15; htes3_2e12; htes3_2f14; htes3_2g7; htes3_2h1; htes3_2h15; htes3_2l19; htes3_2m18; htes3_2m20; htes3_2n9; htes3_2ol3; htes3_30f4; Htes3_35b4; htes3_35b5; htes3_35e21; htes3_35g6; htes3_35k16; htes3_35k24; htes3_35n12; htes3_35n24; htes3_35n9; htes3_35p17; htes3_35p22; htes3_4b4; htes3_4f17; htes3_4f5; htes3_4h6; htes3_4o19; htes3_50j4; htes3_50n06;

htes3_50n23; htes3_6b21; htes3_6c11; htes3_6d16; htes3_72k11; Htes3_72k15; htes3_72p16; htes3_7b22; htes3_7d17; htes3_7j3; htes3_7j8; htes3_7p10; htes3_7p9; htes3_8e24; Htes3_8g11; Htes3_8g5; htes3_8m10; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; hutel_17k7; hutel_18c12; hutel_18i19; hutel_18i4; hutel_18l1; hutel_19f19; hutel_19g19; hutel_19g22; hutel_19h17; hutel_19j11; hutel_1i2; hutel_20b19; hutel_20g21; hutel_20h13; hutel_20m11; hutel_20m24; hutel_21d15; hutel_22d2; hutel_22e12; hutel_22n2; hutel_22o2; hutel_23e13; hutel_23g11; hutel_24c19; hutel_24e11; hutel_24j6; hutel_2h3; their complements; and variants thereof.

2. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_16f21; hfbr2_16g18; hfbr2_16i12; hfbr2_16k22; hfbr2_16l12; hfbr2_22f21; hfbr2_22h13; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23b10; hfbr2_23b21; hfbr2_23f2; hfbr2_23l24; ; hfbr2_23n16; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2b17; hfbr2_2b5; hfbr2_2c1; hfbr2_2c17; hfbr2_2c18; hfbr2_2d15; hfbr2_2d17; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2i17; hfbr2_2k14; hfbr2_2k19; hfbr2_3c18; hfbr2_3f16; hfbr2_3g8; hfbr2_3l2; hfbr2_41m15; hfbr2_62b11; hfbr2_62f10; hfbr2_62l19; hfbr2_62n10; hfbr2_62o17; hfbr2_64a11; hfbr2_64a15; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64j18; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6b24; hfbr2_6i20; hfbr2_6o17; hfbr2_71o20; hfbr2_72b18; hfbr2_72d13; hfbr2_72l12; hfbr2_72m16; hfbr2_72n12; hfbr2_78c24; hfbr2_78d13; hfbr2_78k24; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82c20; hfbr1_10c20; hfbr2_82e17; hfbr1_10e17; hfbr2_82e4; hfbr1_10e4; hfbr2_82g14; hfbr1_10g14; hfbr2_82i17; hfbr1_10; hfbr2_82i24; hfbr1_10; hfbr2_82m16; hfbr1_10; hfbr2_82m6; hfbr1_10; their complements; and variants thereof.

3. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16f21; hfbr2_16k22; hfbr2_22f21; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23f2; ; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2c1; hfbr2_2c18; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2k19; hfbr2_3f16; hfbr2_3l2; hfbr2_62n10; hfbr2_64a11; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64o16; hfbr2_6a17; hfbr2_6i20; hfbr2_71o20;

hfbr2_72d13; hfbr2_72m16; hfbr2_72n12; hfbr2_78d13; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82m16; and hfbr1_10.

4. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfkd2_1j9; hfkd2_24a15; hfkd2_24b15; hfkd2_24e23; hfkd2_24n20; hfkd2_24p5; hfkd2_3i13; hfkd2_3o17; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_46j20; hfkd2_46k19; hfkd2_46m4; hfkd2_47a4; hfkd2_4b6; hfkd2_4c8; hfkd2_4k14; hfkd2_4m11; their complements; and variants thereof.

5. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfkd2_1j9; hfkd2_24e23; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_4b6; hfkd2_4c8; their complements; and variants thereof.

6. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hmcf1_1a11; hmcf1_1c23; hmcf1_1e15; hmcf1_1g13; their complements; and variants thereof.

7. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hmcf1_1c23 hmcf1_1g13; their complements; and variants thereof.

8. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hhtes3_1n3; htes3_14g5; htes3_14h21; htes3_14p14; htes3_14p7; htes3_15a13; Htes3_15c24; htes3_15c6; htes3_15g14; htes3_15h1; htes3_15i5; htes3_15j18; Htes3_15j3; htes3_15k11; htes3_17f10; htes3_17l17; htes3_17n12; htes3_17n18; Htes3_18f3; htes3_18l7; htes3_19f19; htes3_19j17; htes3_1c1; htes3_1g13; htes3_1k11; htes3_20c21; htes3_20k2; htes3_20m18; htes3_21d4; htes3_21j15; htes3_21l16; htes3_21n23; htes3_22c23; htes3_22g2; htes3_22n13; htes3_23l11; htes3_23n19; Htes3_23n19; htes3_26g22; htes3_27d1; htes3_27k4; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2a17; htes3_2d15; htes3_2e12; htes3_2f14; htes3_2g7; htes3_2h1; htes3_2h15; htes3_2l19; htes3_2m18; htes3_2m20; htes3_2n9; htes3_2o13; htes3_30f4; Htes3_35b4; htes3_35b5; htes3_35e21;

htes3_35g6; htes3_35k16; htes3_35k24; htes3_35n12; htes3_35n24; htes3_35n9; htes3_35p17; htes3_35p22; htes3_4b4; htes3_4f17; htes3_4f5; htes3_4h6; htes3_4o19; htes3_50j4; htes3_50n06; htes3_50n23; htes3_6b21; htes3_6c11; htes3_6d16; htes3_72k11; Htes3_72k15; htes3_72p16; htes3_7b22; htes3_7d17; htes3_7j3; htes3_7j8; htes3_7p10; htes3_7p9; htes3_8e24; Htes3_8g11; Htes3_8g5; htes3_8m10; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; their complements; and variants thereof.

9. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: htes3_14g5; htes3_14p14; htes3_14p7; htes3_15a13; htes3_15g14; htes3_15h1; htes3_15j18; htes3_17f10; Htes3_18f3; htes3_19f19; htes3_19j17; htes3_20c21; htes3_21n23; htes3_22c23; htes3_22n13; Htes3_23n19; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2d15; htes3_2f14; htes3_2g7; htes3_2h15; htes3_2l19; htes3_2m20; htes3_2n9; htes3_30f4; htes3_35g6; htes3_35n24; htes3_35p17; htes3_4b4; htes3_4f17; htes3_4o19; htes3_50j4; htes3_50n23; htes3_50n06; htes3_6b21; htes3_6d16; htes3_72k11; htes3_7d17; htes3_7j8; Htes3_8g11; Htes3_8g5; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; their complements; and variants thereof.

10. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16g18; hfbr2_2k14; Htes3_35b4; htes3_35p22; htes3_7j3; htes3_7p10; hutel_20m11; their complements; and variants thereof.

11. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_2b5; htes3_15i5; htes3_18l7; htes3_1k11; Htes3_72k15; htes3_7b22; hutel_19g22; hutel_24j6; their complements; and variants thereof.

12. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_2d15; htes3_35e21; hutel_2h3; their complements; and variants thereof.

13. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_23l24; hfbr2_2i17; hfbr2_41m15; hfbr2_62f10; hfbr2_62l19; hfbr2_64j18;

hfkd2_24n20; hfkd2_24p5; hfkd2_4k14; htes3_1g13; htes3_21l16; htes3_23l11; htes3_26g22; htes3_4h6; htes3_72p16; hutel_19h17; hutel_20h13; hutel_24e11; their complements; and variants thereof.

14. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_3g8; hfbr2_62o17; hfbr2_6b24; hfbr2_78k24; hfkd2_24b15; hfkd2_3o17; hfkd2_46j20; htes3_17l17; htes3_17n18; htes3_27d1; htes3_2a17; htes3_35b5; htes3_35k16; htes3_35n12; htes3_35n9; hutel_20b19; hutel_20m24; hutel_23e13; their complements; and variants thereof.

15. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_23b10; hfbr2_3c18; hfbr2_64a15; hfbr2_6o17; hfbr2_72b18; hfbr2_72l12; hfbr2_82i24(hfbr1_10); htes3_14h21; Htes3_15j3; htes3_20m18; htes3_22g2; htes3_2m18; htes3_7p9; htes3_8m10; hutel_18l1; their complements; and variants thereof.

16. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_23b21; hfbr2_23n16; hfbr2_2c17; hfbr2_62b11; hfbr2_78c24; hfbr2_82e4 (hfbr1_10e4); hfbr2_82i17 (hfbr1_10); hfbr2_82m6 (hfbr1_10); hfkd2_46m4; htes3_15k11; htes3_1c1; hhtes3_1n3; htes3_20k2; htes3_21d4; htes3_23n19; htes3_4f5; htes3_6c11; htes3_8e24; hutel_20g21; hutel_22d2; hutel_22e12; their complements; and variants thereof.

17. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfbr2_16i12; hfbr2_16l12; hfbr2_22h13; hfbr2_2b17; hfbr2_2d17; hfbr2_64k24; hfbr2_82c20 (hfbr1_10c20); hfbr2_82e17 (hfbr1_10e17); hfbr2_82g14 (hfbr1_10g14); hfkd2_24a15; hfkd2_3i13; hfkd2_4m11; hmcf1_1a11; hmcf1_1e15; htes3_15c6; htes3_2ol3; htes3_27k4; htes3_2h1; htes3_35k24; hutel_19f19; and hutel_24c19; their complements; and variants thereof.

18. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hfkd2_46k19; hfkd2_47a4;

htes3_2e12; htes3_21j15; htes3_17n12; hutel_18i19; hutel_1i2; their complements; and variants thereof.

19. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hutel_17k7; hutel_18c12; hutel_18i19; hutel_18i4; hutel_18l1; hutel_19f19; hutel_19g19; hutel_19g22; hutel_19h17; hutel_19j11; hutel_1i2; hutel_20b19; hutel_20g21; hutel_20h13; hutel_20m11; hutel_20m24; hutel_21d15; hutel_22d2; hutel_22e12; hutel_22n2; hutel_22o2; hutel_23e13; hutel_23g11; hutel_24c19; hutel_24e11; hutel_24j6; hutel_2h3; their complements; and variants thereof.

20. An assemblage, comprising at least one nucleic acid molecule having the sequence of a clone selected from the group consisting of: hutel_17k7; hutel_18c12; hutel_18i4; hutel_19g19; hutel_19j11; hutel_22n2; hutel_21d15; hutel_22o2; hutel_23g11; their complements; and variants thereof.

21. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_16f21; hfbr2_16g18; hfbr2_16i12; hfbr2_16k22; hfbr2_16l12; hfbr2_22f21; hfbr2_22h13; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23b10; hfbr2_23b21; hfbr2_23f2; hfbr2_23l24; ; hfbr2_23n16; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2b17; hfbr2_2b5; hfbr2_2c1; hfbr2_2c17; hfbr2_2c18; hfbr2_2d15; hfbr2_2d17; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2i17; hfbr2_2k14; hfbr2_2k19; hfbr2_3c18; hfbr2_3f16; hfbr2_3g8; hfbr2_3l2; hfbr2_41m15; hfbr2_62b11; hfbr2_62f10; hfbr2_62l19; hfbr2_62n10; hfbr2_62o17; hfbr2_64a11; hfbr2_64a15; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64j18; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6b24; hfbr2_6i20; hfbr2_6o17; hfbr2_71o20; hfbr2_72b18; hfbr2_72d13; hfbr2_72l12; hfbr2_72m16; hfbr2_72n12; hfbr2_78c24; hfbr2_78d13; hfbr2_78k24; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82c20; hfbr1_10c20; hfbr2_82e17; hfbr1_10e17; hfbr2_82e4;; hfbr1_10e4; hfbr2_82g14;; hfbr1_10g14; hfbr2_82i17;; hfbr1_10; hfbr2_82i24;; hfbr1_10; hfbr2_82m16;; hfbr1_10; hfbr2_82m6;; hfbr1_10; hfkd2_1j9; hfkd2_24a15; hfkd2_24b15; hfkd2_24e23; hfkd2_24n20; hfkd2_24p5; hfkd2_3i13; hfkd2_3o17; hfkd2_46a6;

hfkd2_46b10; hfkd2_46d13; hfkd2_46j20; hfkd2_46k19; hfkd2_46m4; hfkd2_47a4; hfkd2_4b6; hfkd2_4c8; hfkd2_4k14; hfkd2_4m11; hmcf1_1a11; hmcf1_1c23; hmcf1_1e15; hmcf1_1g13; hhtes3_1n3; htes3_14g5; htes3_14h21; htes3_14p14; htes3_14p7; htes3_15a13; Htes3_15c24; htes3_15c6; htes3_15g14; htes3_15h1; htes3_15i5; htes3_15j18; Htes3_15j3; htes3_15k11; htes3_17f10; htes3_17l17; htes3_17n12; htes3_17n18; Htes3_18f3; htes3_18l7; htes3_19f19; htes3_19j17; htes3_1c1; htes3_1g13; htes3_1k11; htes3_20c21; htes3_20k2; htes3_20m18; htes3_21d4; htes3_21j15; htes3_21l16; htes3_21n23; htes3_22c23; htes3_22g2; htes3_22n13; htes3_23l11; htes3_23n19; Htes3_23n19; htes3_26g22; htes3_27d1; htes3_27k4; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2a17; htes3_2d15; htes3_2e12; htes3_2f14; htes3_2g7; htes3_2h1; htes3_2h15; htes3_2l19; htes3_2m18; htes3_2m20; htes3_2n9; htes3_2o13; htes3_30f4; Htes3_35b4; htes3_35b5; htes3_35e21; htes3_35g6; htes3_35k16; htes3_35k24; htes3_35n12; htes3_35n24; htes3_35n9; htes3_35p17; htes3_35p22; htes3_4b4; htes3_4f17; htes3_4f5; htes3_4h6; htes3_4o19; htes3_50j4; htes3_50n06; htes3_50n23; htes3_6b21; htes3_6c11; htes3_6d16; htes3_72k11; Htes3_72k15; htes3_72p16; htes3_7b22; htes3_7d17; htes3_7j3; htes3_7j8; htes3_7p10; htes3_7p9; htes3_8e24; Htes3_8g11; Htes3_8g5; htes3_8m10; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; hutel_17k7; hutel_18c12; hutel_18i19; hutel_18i4; hutel_18l1; hutel_19f19; hutel_19g19; hutel_19g22; hutel_19h17; hutel_19j11; hutel_1i2; hutel_20b19; hutel_20g21; hutel_20h13; hutel_20m11; hutel_20m24; hutel_21d15; hutel_22d2; hutel_22e12; hutel_22n2; hutel_22o2; hutel_23e13; hutel_23g11; hutel_24c19; hutel_24e11; hutel_24j6; hutel_2h3; their complements; and variants thereof.

22. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_16f21; hfbr2_16g18; hfbr2_16i12; hfbr2_16k22; hfbr2_16l12; hfbr2_22f21; hfbr2_22h13; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23b10; hfbr2_23b21; hfbr2_23f2; hfbr2_23l24; ; hfbr2_23n16; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2b17; hfbr2_2b5; hfbr2_2c1; hfbr2_2c17; hfbr2_2c18; hfbr2_2d15; hfbr2_2d17; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2i17; hfbr2_2k14; hfbr2_2k19; hfbr2_3c18; hfbr2_3f16; hfbr2_3g8; hfbr2_3l2; hfbr2_41m15; hfbr2_62b11; hfbr2_62f10; hfbr2_62l19; hfbr2_62n10; hfbr2_62o17; hfbr2_64a11;

hfbr2_64a15; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64j18; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6b24; hfbr2_6i20; hfbr2_6o17; hfbr2_71o20; hfbr2_72b18; hfbr2_72d13; hfbr2_72l12; hfbr2_72m16; hfbr2_72n12; hfbr2_78c24; hfbr2_78d13; hfbr2_78k24; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82c20; hfbr1_10c20; hfbr2_82e17; hfbr1_10e17; hfbr2_82e4; hfbr1_10e4; hfbr2_82g14; hfbr1_10g14; hfbr2_82i17; hfbr1_10; hfbr2_82i24; hfbr1_10; hfbr2_82m16; hfbr1_10; hfbr2_82m6; hfbr1_10; complements of the nucleic acid sequences; and variants thereof.

23. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16f21; hfbr2_16k22; hfbr2_22f21; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23f2; ; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2c1; hfbr2_2c18; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2k19; hfbr2_3f16; hfbr2_3l2; hfbr2_62n10; hfbr2_64a11; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6i20; hfbr2_71o20; hfbr2_72d13; hfbr2_72m16; hfbr2_72n12; hfbr2_78d13; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82m16; hfbr1_10; complements of the nucleic acid sequences; and variants thereof.

24. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfkd2_1j9; hfkd2_24a15; hfkd2_24b15; hfkd2_24e23; hfkd2_24n20; hfkd2_24p5; hfkd2_3i13; hfkd2_3o17; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_46j20; hfkd2_46k19; hfkd2_46m4; hfkd2_47a4; hfkd2_4b6; hfkd2_4c8; hfkd2_4k14; hfkd2_4m11; complements of the nucleic acid sequences; and variants thereof.

25. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfkd2_1j9; hfkd2_24e23; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_4b6; hfkd2_4c8; complements of the nucleic acid sequences; and variants thereof.

26. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of:

hmcf1_1a11; hmcf1_1c23; hmcf1_1e15; hmcf1_1g13; complements of the nucleic acid sequences; and variants thereof.

27. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hmcf1_1c23; hmcf1_1g13; complements of the nucleic acid sequences; and variants thereof.

28. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hhtes3_1n3; htes3_14g5; htes3_14h21; htes3_14p14; htes3_14p7; htes3_15a13; Htes3_15c24; htes3_15c6; htes3_15g14; htes3_15h1; htes3_15i5; htes3_15j18; Htes3_15j3; htes3_15k11; htes3_17f10; htes3_17l17; htes3_17n12; htes3_17n18; Htes3_18f3; htes3_18l7; htes3_19f19; htes3_19j17; htes3_1c1; htes3_1g13; htes3_1k11; htes3_20c21; htes3_20k2; htes3_20m18; htes3_21d4; htes3_21j15; htes3_21l16; htes3_21n23; htes3_22c23; htes3_22g2; htes3_22n13; htes3_23l11; htes3_23n19; Htes3_23n19; htes3_26g22; htes3_27d1; htes3_27k4; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2a17; htes3_2d15; htes3_2e12; htes3_2f14; htes3_2g7; htes3_2h1; htes3_2h15; htes3_2l19; htes3_2m18; htes3_2m20; htes3_2n9; htes3_2o13; htes3_30f4; Htes3_35b4; htes3_35b5; htes3_35e21; htes3_35g6; htes3_35k16; htes3_35k24; htes3_35n12; htes3_35n24; htes3_35n9; htes3_35p17; htes3_35p22; htes3_4b4; htes3_4f17; htes3_4f5; htes3_4h6; htes3_4o19; htes3_50j4; htes3_50n06; htes3_50n23; htes3_6b21; htes3_6c11; htes3_6d16; htes3_72k11; Htes3_72k15; htes3_72p16; htes3_7b22; htes3_7d17; htes3_7j3; htes3_7j8; htes3_7p10; htes3_7p9; htes3_8e24; Htes3_8g11; Htes3_8g5; htes3_8m10; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; complements of the nucleic acid sequences; and variants thereof.

29. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: htes3_14g5; htes3_14p14; htes3_14p7; htes3_15a13; htes3_15g14; htes3_15h1; htes3_15j18; htes3_17f10; htes3_17n18; Htes3_18f3; htes3_19f19; htes3_19j17; htes3_20c21; htes3_21n23; htes3_22c23; htes3_22n13; Htes3_23n19; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2d15; htes3_2f14; htes3_2g7; htes3_2h15; htes3_2l19; htes3_2m20; htes3_2n9; htes3_30f4; htes3_35g6; htes3_35n24; htes3_35p17; htes3_4b4; htes3_4f17;

htes3_4o19; htes3_50j4; htes3_50n23; htes3_50n06; htes3_6b21; htes3_6d16; htes3_72k11; htes3_7d17; htes3_7j8; Htes3_8g11; Htes3_8g5; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; complements of the nucleic acid sequences; and variants thereof.

30. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16g18; hfbr2_2k14; Htes3_35b4; htes3_35p22; htes3_7j3; htes3_7p10; hutel_20m11; complements of the nucleic acid sequences; and variants thereof.

31. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16c16; hfbr2_2b5; htes3_15i5; htes3_18l7; htes3_1k11; Htes3_72k15; htes3_7b22; hutel_19g22; hutel_24j6; complements of the nucleic acid sequences; and variants thereof.

32. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_2d15; htes3_35e21; hutel_2h3; complements of the nucleic acid sequences; and variants thereof.

33. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_23l24; hfbr2_2i17; hfbr2_41m15; hfbr2_62f10; hfbr2_62l19; hfbr2_64j18; hfkd2_24n20; hfkd2_24p5; hfkd2_4k14; htes3_1g13; htes3_21l16; htes3_23l11; htes3_26g22; htes3_4h6; htes3_72p16; hutel_19h17; hutel_20h13; hutel_24e11; complements of the nucleic acid sequences; and variants thereof.

34. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_3g8; hfbr2_62o17; hfbr2_6b24; hfbr2_78k24; hfkd2_24b15; hfkd2_3o17; hfkd2_46j20; htes3_17l17; Htes3_17n18; htes3_27d1; htes3_2a17; htes3_35b5; htes3_35k16; htes3_35n12; htes3_35n9; hutel_20b19; hutel_20m24; hutel_23e13; complements of the nucleic acid sequences; and variants thereof.

35. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of:

hfbr2_23b10; hfbr2_3c18; hfbr2_64a15; hfbr2_6o17; hfbr2_72b18; hfbr2_72l12; hfbr2_82i24(hfbr1_10); htes3_14h21; Htes3_15j3; htes3_20m18; htes3_22g2; htes3_2m18; htes3_7p9; htes3_8m10; hutel_18l1; complements of the nucleic acid sequences; and variants thereof.

36. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_23b21; hfbr2_23n16; hfbr2_2c17; hfbr2_62b11; hfbr2_78c24; hfbr2_82e4 (hfbr1_10e4); hfbr2_82i17 (hfbr1_10); hfbr2_82m6 (hfbr1_10); hfkd2_46m4; htes3_15k11; htes3_1c1; hhtes3_1n3; htes3_20k2; htes3_21d4; htes3_23n19; htes3_4f5; htes3_6c11; htes3_8e24; hutel_20g21; hutel_22d2; hutel_22e12; complements of the nucleic acid sequences; and variants thereof.

37. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfbr2_16i12; hfbr2_16l12; hfbr2_22h13; hfbr2_2b17; hfbr2_2d17; hfbr2_64k24; hfbr2_82c20 (hfbr1_10c20); hfbr2_82e17 (hfbr1_10e17); hfbr2_82g14 (hfbr1_10g14); hfkd2_24a15; hfkd2_3i13; hfkd2_4m11; hmcf1_1a11; hmcf1_1e15; htes3_15c6; htes3_2ol3; htes3_27k4; htes3_2h1; htes3_35k24; hutel_19f19; and hutel_24c19; complements of the nucleic acid sequences; and variants thereof.

38. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hfkd2_46k19; hfkd2_47a4; htes3_2e12; htes3_21j15; htes3_17n12; hutel_18i19; hutel_1i2; complements of the nucleic acid sequences; and variants thereof.

39. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hutel_17k7; hutel_18c12; hutel_18i19; hutel_18i4; hutel_18l1; hutel_19f19; hutel_19g19; hutel_19g22; hutel_19h17; hutel_19j11; hutel_1i2; hutel_20b19; hutel_20g21; hutel_20h13; hutel_20m11; hutel_20m24; hutel_21d15; hutel_22d2; hutel_22e12; hutel_22n2; hutel_22o2; hutel_23e13; hutel_23g11; hutel_24c19; hutel_24e11; hutel_24j6; hutel_2h3; complements of the nucleic acid sequences; and variants thereof.

40. A computer readable medium, comprising in electronic form at least one nucleic acid or protein sequence of a clone selected from the group consisting of: hutel_17k7; hutel_18c12; hutel_18i4; hutel_19g19; hutel_19j11; hutel_22n2; hutel_21d15; hutel_22o2; hutel_23g11; complements of the nucleic acid sequences; and variants thereof.

41. A nucleic acid molecule having the sequence of a clone selected from the group consisting of hfbr2_16c16; hfbr2_16f21; hfbr2_16g18; hfbr2_16i12; hfbr2_16k22; hfbr2_16l12; hfbr2_22f21; hfbr2_22h13; hfbr2_22h13; hfbr2_22i4; hfbr2_22k3; hfbr2_22k8; hfbr2_23b10; hfbr2_23b21; hfbr2_23f2; hfbr2_23l24; hfbr2_23n16; hfbr2_23o24; hfbr2_23o5; hfbr2_2a2; hfbr2_2b17; hfbr2_2b5; hfbr2_2c1; hfbr2_2c17; hfbr2_2c18; hfbr2_2d15; hfbr2_2d17; hfbr2_2d20; hfbr2_2g18; hfbr2_2h1; hfbr2_2h10; hfbr2_2i17; hfbr2_2k14; hfbr2_2k19; hfbr2_3b16; hfbr2_3c18; hfbr2_3f16; hfbr2_3g8; hfbr2_3l2; hfbr2_41m15; hfbr2_62b11; hfbr2_62f10; hfbr2_62l19; hfbr2_62n10; hfbr2_62o17; hfbr2_64a11; hfbr2_64a15; hfbr2_64c16; hfbr2_64c4; hfbr2_64h6; hfbr2_64i20; hfbr2_64j18; hfbr2_64k24; hfbr2_64o16; hfbr2_6a17; hfbr2_6b24; hfbr2_6i20; hfbr2_6o17; hfbr2_71o20; hfbr2_72b18; hfbr2_72d13; hfbr2_72l12; hfbr2_72m16; hfbr2_72n12; hfbr2_78c24; hfbr2_78d13; hfbr2_78k24; hfbr2_78n23; hfbr2_7a24; hfbr2_7e22; hfbr2_7j4; hfbr2_82c20; hfbr1_10c20; hfbr2_82e17; hfbr1_10e17; hfbr2_82e4;; hfbr1_10e4; hfbr2_82g14;; hfbr1_10g14; hfbr2_82i17;; hfbr1_10; hfbr2_82i24;; hfbr1_10; hfbr2_82m16;; hfbr1_10; hfbr2_82m6;; hfbr1_10; hfkd2_1j9; hfkd2_24a15; hfkd2_24b15; hfkd2_24e23; hfkd2_24n20; hfkd2_24p5; hfkd2_3i13; hfkd2_3o17; hfkd2_46a6; hfkd2_46b10; hfkd2_46d13; hfkd2_46j20; hfkd2_46k19; hfkd2_46m4; hfkd2_47a4; hfkd2_4b6; hfkd2_4c8; hfkd2_4k14; hfkd2_4m11; hmcf1_1a11; hmcf1_1c23; hmcf1_1e15; hmcf1_1g13; hhtes3_1n3; htes3_14g5; htes3_14h21; htes3_14p14; htes3_14p7; htes3_15a13; Htes3_15c24; htes3_15c6; htes3_15g14; htes3_15h1; htes3_15i5; htes3_15j18; Htes3_15j3; htes3_15k11; htes3_17f10; htes3_17l17; htes3_17n12; htes3_17n18; Htes3_18f3; htes3_18l7; htes3_19f19; htes3_19j17; htes3_1c1; htes3_1g13; htes3_1k11; htes3_20c21; htes3_20k2; htes3_20m18; htes3_21d4; htes3_21j15; htes3_21l16; htes3_21n23; htes3_22c23; htes3_22g2; htes3_22n13; htes3_23l11; htes3_23n19; Htes3_23n19; htes3_26g22; htes3_27d1; htes3_27k4; htes3_27o14; htes3_28d14; htes3_2a11; htes3_2a17; htes3_2d15; htes3_2e12; htes3_2f14; htes3_2g7; htes3_2h1; htes3_2h15; htes3_2l19; htes3_2m18;

htes3_2m20; htes3_2n9; htes3_2o13; htes3_30f4; Htes3_35b4; htes3_35b5; htes3_35e21; htes3_35g6; htes3_35k16; htes3_35k24; htes3_35n12; htes3_35n24; htes3_35n9; htes3_35p17; htes3_35p22; htes3_4b4; htes3_4f17; htes3_4f5; htes3_4h6; htes3_4o19; htes3_50j4; htes3_50n06; htes3_50n23; htes3_6b21; htes3_6c11; htes3_6d16; htes3_72k11; Htes3_72k15; htes3_72p16; htes3_7b22; htes3_7d17; htes3_7j3; htes3_7j8; htes3_7p10; htes3_7p9; htes3_8e24; Htes3_8g11; Htes3_8g5; htes3_8m10; Htes3_8p7; Htes3_9e22; Htes3_9i20; Htes3_9k22; hutel_17k7; hutel_18c12; hutel_18i19; hutel_18i4; hutel_18l1; hutel_19f19; hutel_19g19; hutel_19g22; hutel_19h17; hutel_19j11; hutel_1i2; hutel_20b19; hutel_20g21; hutel_20h13; hutel_20m11; hutel_20m24; hutel_21d15; hutel_22d2; hutel_22e12; hutel_22n2; hutel_22o2; hutel_23e13; hutel_23g11; hutel_24c19; hutel_24e11; hutel_24j6; hutel_2h3; their complements; and variants thereof.

42. A polypeptide encoded by the nucleic acid molecule according to claim 41.

43. An antibody or fragment thereof that is capable of binding to a specific portion of the peptide according to claim 42.

44. A pharmaceutical composition, comprising (a) an effective amount of a pharmaceutical agent, wherein said pharmaceutical agent is selected from the group consisting of the polypeptide according to claim 42, variants or functional derivatives thereof, and antibodies thereto; and (2) a physiologically acceptable carrier or excipient.

45. An expression vector comprising the nucleic acid molecule of claim 41 or a fragment thereof, and optionally a promoter operably linked to said nucleic acid molecule or said fragment.

46. A method for recombinantly producing a desired peptide, comprising expressing in a host cell a peptide encoded by the nucleic acid molecule according to claim 41.